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 that 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 therapy; 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).

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. In many cases, scientific substantiation of a specific health claim was judged insufficient or based on an indirect effect. 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), 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). 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’. 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. 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, vaginal, respiratory tract, and in the buccal cavity.

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. 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. 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. With the advent of these newer metagenomic tools, the role of probiotics in the restoration of normal microbiota is being revisited.

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. 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) or by indirect methods (Nugent scores). 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.

**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). 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, includingAAD,9 36 37 CDI,9 38 IBD,39 IBS,40 TD,41 eradication ofH. pylori,36 37 BV42 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-
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. Engelbrekston 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, 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.

| 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.
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). Orhage et al had two treatment arms (Bifido longum alone and a mixture of B. longum and L. acidophilus). Larsen et al tested two single probiotics (B. lactis and L. acidophilus) in separate treatment arms. Lidbeck et al 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-cultivable bacterial strains, which included metagenomic assays (FISH, TRFLP, 16s 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.

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 significantly vary by the model of study design ($\chi^2=2.3$, $p=0.32$). 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). 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

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.
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 L. 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.92 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.

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,96 but only showed indirect evidence of short-chain fatty acid changes in the other study95 (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 trials74 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 treatment79 and one found a reduction in bacteroides species80 (Strength: strong, Quality: moderate). Three clinical trials determined there were no significant changes due to Lactobacillus plantarum 299v92–94 (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.95 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.97 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

**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 35 50–58 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 postprobiotic follow-up period.
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*82          | 19                            | FISH                 | Paediatric cancer patients | Chemotherapy             | 8                               | Enhances anaerobes       | No change           |
| *Bifido longum BB536* | Orrhage *et al*62       | 10                            | Culture              | Healthy volunteers | Clindamycin              | 0                               | Restores               | Restores            |
| *Clostridium butyricum* MIYAIRI | Seki *et al*84     | 83                            | Culture              | Paediatric respiratory or GI infections | Antibiotics             | 0                               | Restores               | Restores            |
| *Lactobacillus acidophilus* NCFB1748 | Lidbeck *et al*84   | 5                             | Culture              | Healthy volunteers | Enoxacin or             | 1                               | Restores only in enoxacin | Restores only in enoxacin |
| *Lactobacillus rhamnosus* GG | Zoppi *et al*61   | 7                             | Culture              | Paediatric respiratory infections | Ceftriaxone             | 0                               | Partially corrects      | No change in clindamycin | Partially restores   |
| *Saccharomyces boulardii* lyo | Zoppi *et al*61  | 6                             | Culture              | Paediatric respiratory infections | Ceftriaxone             | 0                               | Improves               | Partially restores    |
| *L. acidophilus+Bifido bifidum* | Black *et al*65      | 10                            | Culture              | Healthy volunteers | Ampicillin               | 2                               | Recovers more rapidly    | Restores            |
|                                         | Zoppi *et al*61          | 7                            | Culture              | Pediatric respiratory infections | Ceftriaxone             | 0                               | Less change            | Partially restores    |
| *L. acidophilus 1748+B. longum BB536* | Orrhage *et al*62   | 10                            | Culture              | Healthy volunteers | Clindamycin              | 0                               | No change               | No change            |
| *L. rhamnosus+L. bifidus+L. acidophilus* | Zoppi *et al*61      | 7                             | Culture              | Paediatric respiratory infections | Ceftriaxone             | 0                               | Partially corrects      | Partially restores    |
| *L. acidophilus 1748+B. animalis lactis* | Jernberg *et al*66     | 4                             | Culture              | Healthy volunteers | Clindamycin              | 2                               | Restores               | Restores            |
| *L. acidophilus 1748+B. lactis Bi-07* | Madden *et al*87      | 15                            | Culture              | *Helicobacter pylori*+H. pylori | Amoxicillin+metronidazole | 2                               | Restores               | Restores            |
| *L. acidophilus 1748+B. animalis lactis* | Jernberg *et al*66     | 4                             | Culture              | Healthy volunteers | Clindamycin              | 2                               | Partially restores      | Restores            |
| *L. acidophilus 1748+B. lactis Bi-07* | Madden *et al*87      | 15                            | Culture              | *Helicobacter pylori*+H. pylori | Amoxicillin+metronidazole | 2                               | Restores               | Restores            |
| *L. acidophilus 1748+B. lactis Bi-07* | Jernberg *et al*66     | 4                             | Culture              | Healthy volunteers | Clindamycin              | 2                               | Partially restores      | Restores            |
| *L. acidophilus 1748+B. lactis Bi-07* | Madden *et al*87      | 15                            | Culture              | *Helicobacter pylori*+H. pylori | Amoxicillin+metronidazole | 2                               | Restores               | Restores            |

*Including strain (when reported).

GI, gastrointestinal.

**McFarland LV. BMJ Open 2014;4:e005047. doi:10.1136/bmjopen-2014-005047. Open Access on September 28, 2023 by guest. Protected by copyright.http://bmjopen.bmj.com/ BMJ Open: first published as 10.1136/bmjopen-2014-005047 on 25 August 2014. Downloaded from**
| 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. | 46 | FISH | Atopic dermatitis | 0 | Modulates NF | No change | – |
| Bifido lactis Bi-07 | Larsen et al. | 17 | PCR | Atopic dermatitis | 0 | No change | No change | – |
| Bifido longum BB536 | Odamaki et al. | 22 | TRFLP | Cedar pollen allergy | 4 weeks | Maintains NF | No change | – |
| Escherichia coli Nissle | Lata et al. | 22 | Culture | Liver cirrhosis | 0 | Restores | Improves | More Bifido and Lactobacillus |
| Lactobacillus acidophilus 700396 | Larsen et al. | 17 | PCR | Atopic dermatitis | 0 | No change | No change | – |
| Lactobacillus casei rhamnosus Lcr35 | Petricevic and Witt | 83 | Nugent scores | Bacterial vaginosis | 4 weeks | Restores | Improves | Improved Nugent scores |
| Lactobacillus plantarum 299v | Nobaek et al. | 25 | Culture | IBS | 4 weeks | No change | No change | – |
| | Klarin et al. | 17 | Culture | Enterally-fed | 0 | No change | No change | – |
| | Klarin et al. | 22 | Culture | Antibiotics | 0 | No change | No change | – |
| Saccharomyces boulardii lyo | Girard et al. | 10 | Culture | Enteral diarrhoea | 3 days | Improves | Improves | – |
| L. rhamnosus GR-1+Lactobacillus fermentum RC14 | Reid et al. | 33 | Nugent scores | Bacterial vaginosis | 2 weeks | Restores | Improves | Improved Nugent scores |
| L. rhamnosus GR-1+L. fermentum RC14 | Reid et al. | 31 | Nugent scores and culture | Bacterial vaginosis | 30 days | Restores | Improves | Improved Nugent scores |
| Lactobacillus plantarum 8PA3 +Bifido bifidum | Kirpich et al. | 32 | Culture | Colon cancer | 0 | Restores | Improves | More E. coli and enterococci |
| L. rhamnosus GR1+Lactobacillus reuteri RC14 | Hummelen et al. | 23 | Nugent score | Bacterial vaginosis | 0 | No change | No change | – |
| L. casei Shiroti+B. breve BBG01 | Uchida et al. | 4 | Culture | Short bowel syndrome | 0 | No change | No change | – |
| L. brevis CD2+Lactobacillus salivarius FV2+L. plantarum FV9 | Mastromarino et al. | 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. | 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. | 41 | PCR | IBS | 0 | Restores | Improves | Improved similarity index |
| | Lyra et al. | 22 | PCR | IBS | 0 | Alters | Alters | More clostridia and Ruminococcus |
| | | | | | | | | | Continued |
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 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. 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. 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. cultured seven healthy volunteers and found enterobacteriaceae and Clostridial species post-B. longum was reduced by 10^4/g compared to baseline (p<0.03), but no other changes in the microbiota were detected. Karlsson et al. 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. Kubota et al. 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).

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. 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. 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. claimed that the mixture of four lactobacilli strains and three bifidobacteria strains ‘modulated vaginal

| Table 2 Continued |
|-------------------|
| Probiotic*         | Reference |
| L. acidophilus+L. paracasei+Lactobacillus delbrueckii spp+bilgaricus+L. plantarum+B. longum+B. infantis+B. breve | Wong et al.  |
| PCR                | 7         |
| 14917               |           |
| Culture            | 20        |
| Culture            | 10        |
| Culture and PCR    | 10        |
| Culture            | 10        |
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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-treatment | Claims stated in papers | Evidence-based claim |
|------------|-----------|-------------------------------|----------------------|---------------------|---------------------------|-------------------------|------------------------|---------------------|
| Bifido animalis lactis DN173010 | Rochet et al$^{R9}$ | 12 | FISH | Healthy | None | 10 days | No change | No change |
| | Oswari et al$^{R1}$ | 160 | PCR | Volunteers | None | 6 months | No change | No change |
| Bifido bifidum | Langhendries et al$^{R2}$ | 20 | Culture | Healthy volunteers | None | 0 | No change | No change |
| Bifido longum | Benno and Mitsuoka$^{R3}$ | 5 | Culture | Healthy volunteers | None | 0 | No change | No change |
| | Fujiwara et al$^{R4}$ | 7 | Culture | Healthy volunteers | None | 30 days | Alters | Alters |
| | Harmsen et al$^{R5}$ | 14 | FISH | Volunteers | None | 0 | No change | No change |
| Lactobacillus casei ND114001 | Guerin et al$^{R6}$ | 12 | FISH | Healthy volunteers | None | 10 days | No change | No change |
| | Rochet et al$^{R7}$ | 12 | FISH | Healthy volunteers | None | 10 days | No change | No change |
| | Rochet et al$^{R8}$ | 7 | FISH | Healthy volunteers | None | 0 | No change | No change |
| Lactobacillus johnsonii La1 | Brunser et al$^{R9}$ | 32 | Culture and FISH | Healthy volunteers | None | 2 weeks | No claim | No change |
| Lactobacillus plantarum 299v | Goossens et al$^{R10}$ | 11 | Culture | Healthy | None | 3 weeks | No change | No change |
| | Goossens et al$^{R11}$ | 32 | Culture | Healthy | None | 4 weeks | No change | No change |
| | Goossens et al$^{R12}$ | 15 | Culture | Colonic | None | 0 | No change | No change |
| | Berggren et al$^{R13}$ | 33 | Culture | Polyps | None | 0 | No change | No change |
| | Karlsson et al$^{R14}$ | 9 | TRFLP | Healthy, atherosclerosis | None | 0 | No change | No change |
| Lactobacillus rhamnosus GG | Gueimonde et al$^{R15}$ | 29 | PCR | Healthy volunteers | None | 0 | No change | No change |
| Lactobacillus salivarius CECT5713 | Sierra et al$^{R16}$ | 20 | Culture | Healthy volunteers | None | Improves | No change | No change |
| Saccharomyces boulardii lyo | Vanhoutte et al$^{R17}$ | 30 | PCR | Healthy volunteers | None | 0 | No change | No change |
| B. animalis+B. longum | Zhong et al$^{R18}$ | 11 | FISH | Healthy volunteers | None | 7 days | No change | No change |
| | He et al$^{R19}$ | 11 | FISH | Healthy volunteers | None | 7 days | Modifies | No change |
| L. acidophilus+B. lactis | Yang and Sheu$^{R20}$ | 63 | Culture | Healthy but 55% H. pylori+ | None | 0 | Restores | Alters |
| | Mah et al$^{R21}$ | 20 | FISH | Healthy neonates | None | 6 months | No change | No change |
| L. rhamnosus GG+B. longum Bb536 | Rafter et al$^{R22}$ | 38 | Culture | Patients with colon cancer or at risk | None | 0 | No change | No change |

Continued
| Probiotic* | Reference            | Number 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 |
|-----------|----------------------|-------------------------------|----------------------|-----------------------------------|--------------------------|--------------------------|-------------------------|-----------------------|
| *Including strain (when reported). FISH, fluorescence in situ hybridisation analysis; TRFLP, terminal restriction fragment length polymorphism analysis.

| Probiotic | Reference | Number 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 |
|-----------|-----------|-------------------------------|----------------------|-----------------------------------|--------------------------|--------------------------|-------------------------|-----------------------|
| L. rhamnosus GG+Lactobacillus gasseri TMC0356 | Kubota et al¹⁰³ | 14 | Culture FISH TRFLP | Healthy, allergy patients | None | 0 | Suppressed changes | Alters |
| L. paracasei B21060+L. paracasei B21070+L. gasseri B21090 | Morelli et al¹⁰⁴ | 12 | Culture Culture | Healthy volunteers | None | 3 days | No claims | No change |
| L. acidophilus 1748+L. paracasei F19+B. lactis Bb12 | Sullivan et al¹⁰⁵ | 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 al¹⁰⁶ | 22 | Culture TRFLP PCR | Healthy volunteers | None | 2 weeks | No change | No change |
| B. animalis lactis+Lactobacillus delbrueckii l-1632+L. delbrueckii l-1519+L. lactis cremoris | McNulty et al¹⁰⁶ | 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 al¹⁰⁴ | 15 | PCR | Healthy pregnant volunteers | None | 0 | Modulates | No change |
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 and the American Society for Nutrition. 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. 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. 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
| Probiotic* | Restored normal microbiota* | Altered normal microbiota* | Ranked net evidence for efficacy† | Vaginitis/BV | Acute paediatric diarrhoea |
|-----------|-----------------------------|-----------------------------|----------------------------------|-------------|--------------------------|
|           |                             |                             | AAD | CDI | IBD | IBS | TD | H pylori | BV |                         |
| Restores microbiota |                             |                             |  |     |     |     |     |         |     |                         |
| Clostridium butyricum MIYAIRI | Yes | ND | – | – | – | – | – | – | – | – | – | – |
| Lactobacillus. acidophilus+Bifido bifidum | Yes | ND | 0 | – | – | – | – | – | – | – | – | – |
| L. acidophilus 1748+Lactobacillus paracasei F19+Bifido lactis Bb12 | Yes | ND | – | + | – | – | – | – | – | – | – | – |
| Bifido longum | Yes | ND | – | – | – | – | – | – | – | – | – | – |
| 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 | 0 | ++ | – | ++ | 0 |
| L. acidophilus | Partial | No | ++ | ++ | ++ | – | – | – | + | 0 | – | – |
| L. acidophilus+B. bifidum+L. rhamnosus | Partial | ND | – | – | – | – | – | – | – | – | – | – |
| Alters microbiota |                             |                             |  |     |     |     |     |         |     |                         |
| Escherichia coli Nissle | ND | Yes | – | – | – | – | – | – | – | – | – | – |
| L. casei (DN114001 or Lcr35) | ND | Yes | + | – | 0 | + | ++ | ++ | – | ++ | 0 | ++ |
| L. rhamnosus GR1+Lactobacillus fermentum RC14 | ND | Yes | – | – | 0 | 0 | 0 | 0 | ++ | – | ++ | 0 |
| L. plantarum 8PA3+B. bifidum | ND | Yes | – | – | 0 | 0 | 0 | 0 | ++ | – | ++ | 0 |
| Lactobacillus rhamnosus GG+L. rhamnosus Lc705+P. freudenreichii shermanii JS+Bifido breve Bb99 | ND | Yes | ++ | ++ | ++ | – | – | – | – | – | – | – |
| L. acidophilus+L. plantarum+L. rhamnosus+B bifidum | ND | Yes | – | – | 0 | 0 | 0 | 0 | ++ | – | ++ | 0 |
| Lactobacillus brevis CD2+Lactobacillus. salivarius FV2+L. plantarum FV9 | ND | Yes | – | – | 0 | 0 | 0 | 0 | ++ | – | ++ | 0 |
| L. acidophilus+L. paracasei+Lactobacillus delbrueckii spp. bulgaricus+L. plantarum, Bifido longum, Bifido infantis, Bifido breve | ND | Yes | – | – | 0 | 0 | 0 | 0 | ++ | – | ++ | 0 |
| No effect on microbiota |                             |                             |  |     |     |     |     |         |     |                         |
| Bacillus clausii | ND | ND | – | – | – | – | – | – | – | – | – | – |
| L. plantarum 299v | ND | No | – | – | – | – | – | – | – | – | – | – |
| B. lactis | ND | No | – | – | – | – | – | – | – | – | – | – |
| B. breve | ND | No | – | – | – | – | – | – | – | – | – | – |
| L. acidophilus+B. longum | ND | No | – | – | – | – | – | – | – | – | – | – |
| L. rhamnosus 19070-2+L. reuteri DSM | ND | No | – | – | – | – | – | – | – | – | – | – |
| 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 | – | – | – | – | – | – | – | – | – | – |
| B. bifidum MIMBBb75 | 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. 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.\textsuperscript{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.\textsuperscript{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 assay 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 (\textit{B. longum}, \textit{Clost. butyricum}, \textit{L. acidophilus}, \textit{L. rhamnosus} and \textit{S. boulardii}) and five probiotic mixtures ((\textit{L. acidophilus}+\textit{B. bifidum}), (\textit{L. rhamnosus}+\textit{B. bifidus}+\textit{L. acidophilus}), (\textit{L. acidophilus}+\textit{L. paracasei}+\textit{B. lactis}), (\textit{L. acidophilus}, 2 strains, \textit{B. bifidum}, \textit{B. animalis}) and (\textit{L. acidophilus}+\textit{L. paracasei}+\textit{B. bifidus}+\textit{2 strains of B. lactis})) documented either complete or partial recovery of normal microbiota (model A). Only two probiotic mixtures ((2 strain mixture: \textit{L. acidophilus}+\textit{B. bifidum}) and (4 strain mixture: \textit{L. acidophilus}, 2 strains, \textit{B. bifidum}, \textit{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 (\textit{E. coli Nissle}, \textit{S. boulardii} and \textit{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: \textit{s. boulardii}, a four strain mixture (2 strains of \textit{L. rhamnosus}+\textit{P. freudenreichii}+\textit{B. breve}) and a seven strain mixture (4 lactobacilli and 3 bifidobacteria strains), but only one had consistent results showing improvements in the microbiota.\textsuperscript{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: \textit{B. clausii}, \textit{B. infantis}, \textit{L. brevis}, \textit{L. reuteri}, mix of two strains (\textit{L. acidophilus}+\textit{L. helveticus}), mix of two strains (\textit{L. acidophilus}+\textit{L. casei}) or (\textit{L. acidophilus}+\textit{B. animalis}), mix of four strains (\textit{L. rhamnosus} (two strains), \textit{P. freudenreichii}+\textit{B. animalis}) and mix of seven strains (\textit{L. sporogenes}, \textit{B. bifidum}, \textit{L. bulgaricus}, \textit{L. thermophilus}, \textit{L. acidophilus}, \textit{L. casei}, \textit{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’.\textsuperscript{3 29 112} Donovan et al\textsuperscript{8} 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.20 112 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 strain 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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