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TITLE PAGE

Title: Antibiotics and Probiotics for Irritable Bowel Syndrome.

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ABSTRACT

Irritable bowel syndrome (IBS) is a disorder of gut-brain interaction characterised by abdominal pain and a change in stool form or frequency. Current symptom-based definitions and the classification of IBS promotes heterogeneity amongst patients meaning that there may be several different pathophysiological abnormalities leading to similar symptoms. Although our understanding of IBS is incomplete, there are several indicators that the microbiome may be involved in a subset of patients. Techniques including faecal sample analysis, colonic biopsies, duodenal aspirates, or surrogate markers, such as breath testing, have been used to examine the gut microbiota in individuals with IBS. Because of a lack of a clear definition of what constitutes a healthy gut microbiota, and the fact that alterations in gut microbiota have only been shown to be associated with IBS, a causal relationship is yet to be established. We discuss several hypotheses as to how dysbiosis may be responsible for IBS symptoms, as well as potential treatment strategies. We review the current evidence for the use of antibiotics and probiotics to alter the microbiome in an attempt to improve IBS symptoms. Rifaximin, a non-absorbable antibiotic, is the most studied antibiotic and has now been licensed for use in IBS with diarrhoea in the USA, but the drug remains unavailable in many countries for this indication. Current evidence also suggests that certain probiotics, including *Lactobacillus plantarum DSM 9843* and *Bifidobacterium bifidum MIMBb75*, may be efficacious in some patients with IBS. Finally, we describe the future challenges facing us in our attempt to modulate the microbiome to treat IBS.

Key points

- There are several indicators that the gut microbiota may be implicated in pathophysiology of irritable bowel syndrome (IBS).
- This review discusses the microbiome as a potential therapeutic target for IBS and the current evidence for the use of antibiotics and probiotics as treatments for IBS.

INTRODUCTION

Irritable bowel syndrome (IBS) is a common functional gastrointestinal disorder, now known as a disorder of gut-brain interaction, which affects between 5% and 10% of the world's population.[1, 2] It is characterised by recurrent abdominal pain in association with a change in stool form or frequency.[3] The pathophysiology is complex and incompletely understood meaning that the cause of IBS is unknown and there is currently no available biomarker to diagnose the condition.[4] Instead, a diagnosis of IBS is made using self-reported symptom clusters, proposed by consensus among experts, after limited investigation and in the absence of red flags.[5, 6] The latest symptom-based diagnostic criteria for IBS, the Rome IV criteria, require individuals to have at least weekly abdominal pain with a change in stool form and/or frequency and symptom duration for at least 6 months.[7] Patients are subtyped depending on predominant stool form using the Bristol Stool Form Scale (BSFS): IBS with constipation (IBS-C), IBS with diarrhoea (IBS-D), IBS with mixed bowel habits, or IBS unclassified.

For most people, IBS is chronic and runs a relapsing and remitting course.[8] Although it does not seem to confer an excess mortality risk,[9, 10] its impact should not be underestimated. Quality of life of those with IBS is similar to those with organic gastrointestinal disorders such as Crohn's disease,[11] and other chronic medical conditions such as stroke, chronic obstructive pulmonary disease, or leg ulcers.[12] IBS affects various aspects of daily life such as work productivity,[13, 14, 15, 16] social functioning,[13, 15, 17, 18] and psychological health.[19, 20, 21] The healthcare costs of IBS are substantial with annual estimated costs up to £2 billion in the UK,[22] €8 billion in Europe,[23] ¥123 billion in China,[24] and at least US$10 billion in the USA.[25] These costs have important implications, not for only healthcare systems, but also for clinicians and insurance providers, as well as patients who are willing to forgo their own money to pay for medications to

improve IBS symptoms.[26, 27, 28] Despite all this, there remains, even among clinicians, a perception that IBS lacks an "organic" cause, which attracts negative attitudes or perception towards patients with IBS, resulting in stigma for patients.[29, 30]

At present, IBS is not curable. Current treatment approaches focus on alleviating predominant gastrointestinal symptom(s),[31, 32] and do not change the natural history of the disorder.[8] Most treatments for IBS are of limited efficacy when assessed in randomised controlled trials (RCTs),[33, 34, 35, 36, 37] with high placebo response rates.[38] This is partly because of the heterogenicity among individuals with IBS, meaning that there may be several underlying pathophysiological processes giving rise to similar gastrointestinal symptoms of abdominal pain and altered bowel habits, even among those with the same subtype. These include motility disturbances, visceral hypersensitivity, altered mucosal and immune function, central nervous processing, and perturbations in the gut microbiota.[4]

The role of the latter in IBS has attracted considerable scientific interest for a number of reasons. Firstly, the best known aetiological factor among patients with IBS is the development of symptoms following an acute enteric infection,[39, 40] which is commonly referred to as post-infection IBS (PI-IBS). The faecal microbiota of those with PI-IBS is different from that of healthy controls but similar to those with IBS-D, suggesting that PI-IBS and IBS-D may share a common pathophysiology.[41] Secondly, a large proportion of patients with IBS experience meal-related symptoms and IBS symptoms are often triggered by specific food items. Since diet is known to modify the gut microbiome, even in the short term,[42] this raises the possibility of the microbiome being involved in IBS. Thirdly, although use of broad spectrum antibiotics may also be associated with the development of IBS,[43] rifaximin, an antibiotic which remains largely unabsorbed by the gut, leads to improvement of IBS symptoms in some patients,[44, 45] suggesting that changes in the composition of the gut microbiota may be implicated. Fourthly, accepting the limitations of

obtaining an accurate diagnosis of small intestinal bacterial overgrowth (SIBO) due to imprecision of available tests for the condition, SIBO has been reported to be associated with IBS,[46, 47, 48] and treatment for presumed SIBO leads to an improvement in symptoms.[49] Finally, faecal microbiota transplantation (FMT) may be beneficial in the treatment of IBS,[50] with symptom improvement sustained up to 3 years in one RCT.[51]

However, even though there are several indicators that the gut microbiota may be implicated in pathophysiology of IBS, and the rapid advances made in examining the microbiota,[52] there is still no clear evidence of a specific, reproducible, microbial profile of individuals with IBS.[53, 54] In this review, we will examine how gut microbial dysbiosis may contribute to IBS, discuss the potential of the microbiome as a therapeutic target, and the current evidence for the use of antibiotics and probiotics as treatments for IBS.

THE MICROBIOME

The terms microbiome and microbiota are often used interchangeably but are strictly different. The microbiota refers to a collection of microorganisms, such as bacteria, viruses, fungi, and protozoa, present in a defined environment, whereas the microbiome encompasses the whole ecological community including the microorganisms, their structural elements such as their proteins, lipids, and genetic material, microbacterial metabolites such as signalling molecules or toxins, and the environmental conditions in which they coexist.[55] Trillions of microorganisms are present in the human gut, weighing between 1.5-2.0 kg,[56] and representing more microbial cells in the gut than human cells present in the body.[57] They are considered to be important in maintaining health,[58, 59] and a change in the normal composition or function of the microbiome have been associated with several gastrointestinal disorders such as IBS,[41, 60] inflammatory bowel disease,[61, 62] and colorectal cancer,[63, 64] as well as a range of other medical conditions including diabetes mellitus, obesity, chronic kidney disease, and Parkinson's disease.[65, 66, 67, 68, 69]

In terms of the taxonomic classification, the gut microbiota is composed of twelve phyla, but most are from four dominant phyla namely *Actinobacteria*, *Bacteroidetes*, *Firmicutes*, and *Proteobacteria*.[70] Within those phyla, there are hundreds of species, and their relative composition vary among individuals. Each individual hosts a unique set of at least 160 species in their gut.[56] *Firmicutes*, the largest phylum, consists of key genera like *Clostridium, Lactobacillus*, and *Ruminococcus*, whereas the second largest phylum, *Bacteroidetes,* include the genera *Bacteroides. Bifidobacterium* form part of the *Actinobacteria* phyla and *Escherichia* are included in the *Proterobacteria* phyla.[71] The microbiome is a dynamic environment. The composition of the microbiome changes with age,[72] ethnicity,[73] geographical location,[72, 74] diet,[75, 76, 77], birth mode,[77, 78],

breastfeeding,[79] medication use,[80] household circumstances, including living in close proximity with genetically unrelated individuals,[81] extended family members, or pets, and other lifestyle factors such as exercise,[82] alcohol consumption,[83] or cigarette smoking.[84] (Figure 1)

Gut microorganisms constantly interact with one another.[85] Although not fully understood, microorganisms, through direct interaction or secondary metabolites, may have corporative, neutral, or competitive interactions.[55] Humans and their gut microbiota live in symbiosis, i.e. the human gut provides an environment for the microorganisms to survive, where they assist the human body in performing several key functions. For example, the microbiota play an important role in fermenting undigested substances such as dietary fibre. This supports the growth of some microbacteria that produce short chain fatty acids (SCFAs), the main ones being acetate, propionate, and butyrate. Acetate is absorbed and plays a central role in cholesterol metabolism and lipogenesis and may be involved in appetite regulation.[86] Propionate regulates gluconeogenesis in the liver, whereas butyrate seems to control gut hormones and induces apoptosis of colon cancer cells. [87, 88, 89] Gut microbial enzymes are essential in maintaining bile acid homeostasis in humans.[90] With a central role in many key processes in the human body, the gut microbiome, when altered, may be responsible for ill health. For example, a Clostridia-rich microbiota is associated with excess faecal bile acids in those with IBS-D.[91] The production of trimethylamine-N-oxide is dependent on the metabolism of dietary phosphatidylcholine from meat by the gut microbiota and, because increased trimethylamine-N-oxide levels are associated with major cardiovascular events, the gut microbiota may play a role in the pathophysiology of cardiovascular disease.[92]

Dysbiosis

Dysbiosis refers to a change or deviation from a normal microbiota.[93] Because of the complexity and the variability of the microbiome, there is no definition of a normal healthy gut microbiota and, hence, no gold standard technique to define dysbiosis (Figure 2).[94] As a result, most studies compare the microbiota of individuals of interest with controls to calculate a dysbiosis index. There are several dysbiosis indexes based on different methodologies, which have been reviewed elsewhere.[94] Dysbiosis indexes have simplified the comparison between gut microbiota and have enabled able us to characterise the microbiota in various medical conditions.

Gut microbial dysbiosis has been reported in IBS. One systematic review of case-control studies reported that family *Enterobacteriaceae,* family *Lactobacillaceae,* and genus *Bacteroides* were increased in patients with IBS, whereas genus *Faecalibaterium* and genus *Bifidobacterium* were decreased.[60] Another systematic review demonstrated a reduction in *Lactobacillus* and *Bifidobaterium*, an increase in *Escherichia coli* (family *Enterobacteriacea*), and a marginal increase in family *Enterobacteriacea* among those with IBS.[95] It also demonstrated no difference in *Bacteroides* and *Enterococcus* between those with IBS and healthy controls. The differences in these results may be explained partially by the fact that the latter systematic review only included studies with faecal samples, whilst the former included studies that collected faecal and/or mucosal samples. In fact, the luminal microbiota is different from the mucosal microbiota in both healthy individuals and those with IBS.[96, 97, 98, 99] Most research studies use faecal material to analyse the microbiota,[60] which is easily collected without invasive testing, but may not be an accurate representation of the gut microbiota.

Apart from analysing the microbiota in stool samples, colonic biopsies, or using surrogate markers for microbiome dysbiosis such as lactulose breath testing, other techniques

have been used. Some investigators have obtained duodenal aspirates at upper gastrointestinal endoscopy and demonstrated a change in the small bowel microbiota in individuals with IBS compared with controls.[100, 101, 102]. However, variability in the microbiota occurs in different regions of the gut in health,[96] and as previously discussed, in IBS. Although IBS symptoms are traditionally viewed as colonic in origin, it is unclear whether dysbiosis in one specific area of the gut is more important than in others. In a study using simultaneous breath carbon dioxide, hydrogen, methane, and hydrogen sulphide testing, together with stool microbiome analysis, a distinct gut microbiota was demonstrated in 171 individuals with IBS-C or IBS-D.[103] Those with IBS-C had increased methane on breath testing, which correlated with higher levels of stool methanogens, such as *Methanobrevibacter smithii*, whereas those with IBS-D had increased hydrogen on breath testing, which correlated with higher levels of hydrogen-producing bacteria, including *Fusobacterium* and *Desulfovibrio spp.* These are interesting observations, as human cells are not known to produce either methane or hydrogen,[104] meaning that increased levels of these gases likely originate from the gut microbiota. Although a clear causality between these alterations in the microbiota and predominant stool pattern has yet to be demonstrated, a RCT recruiting 31 individuals with IBS-C with a positive methane breath test demonstrated that those with lower levels of methane after antibiotic treatment had a significantly greater improvement in constipation.[105]

There are several hypotheses that could explain how dysbiosis may be responsible for IBS symptoms. For example, *Lactobacillus* (family *Lactobacillae*) can breakdown fructose or glucose to produce organic acids,[106] associated with abdominal pain and bloating in IBS.[107] The relative increase in family *Enterobacteriacea*, including *Escherichia coli*, may be secondary to previous infection giving rise to low grade inflammation or intestinal dysmotility, seen especially in PI-IBS.[108] However, these are only hypotheses. Altered gut

microbiota has only been associated with IBS; a causal relationship is yet to be established. Dysbiosis may, for instance, be the result of other factors like a change in diet, medication use, gut dysmotility, or altered gut immune system in patients with IBS, rather than a cause of IBS. As a result of all these uncertainties, testing for dysbiosis and SIBO is not recommended in clinical practice.[109]

The microbiome as a potential therapeutic target for the treatment of IBS

Given the accumulating evidence that the microbiota may be implicated in IBS, modulating the microbiome has been of particular interest to many investigators. A diet low in low fermentable oligosaccharides, disaccharides, monosaccharides, and polyols (FODMAPs) has been demonstrated to reduce global IBS symptoms in RCTs.[35] This improvement in symptoms, as demonstrated in a recent study analysing the clinical response and changes in microbiota in patients with IBS and a household control, may be because a low FODMAP diet induces a shift of the microbiota profile in some patients with IBS towards one similar to that of individuals in the control group.[110] These results also suggest that microbial signatures could be useful biomarkers to identify those with IBS who will respond well to a low FODMAP diet. Others have altered the gut microbiota using FMT in an attempt to improve IBS symptoms but had varying results.[51, 111, 112] Similarly, the microbiome has been the main target for other investigators using antibiotics or probiotics in order to alter the gut microbiome profile in an attempt to relieve symptoms of IBS.

ANTIBIOTICS FOR IRRITABLE BOWEL SYNDROME

Although the benefits of antibiotics in the treatment of infectious diseases cannot be understated, our understanding of their effects on the gut microbiome is still limited. A recent systematic review of 129 studies which investigated this issue demonstrated substantial, and sometimes persisting, effects of antibiotics on the composition of the gut microbiota.[113] The clinical consequences of a change in gut microbiota following antibiotics are largely unknown. One retrospective study recruiting over 26,000 individuals showed that exposure to antibiotics may be associated with new onset of IBS.[43] In addition, two population-based studies demonstrated that IBS development was independently associated with any antibiotic use (odds ratio (OR) = 1.80; 95% confidence interval (CI) 1.00 to 3.20),[114] or antibiotics for non-gastrointestinal infections (OR = 2.30; 95% CI 1.22 to 4.33).[115] On the other hand, the increased recognition that the microbiome may play a role in the pathogenesis of IBS, as well as reports that some patients with IBS may have SIBO, and that its treatment leads to an improvement in symptoms, has led to RCTs studying the efficacy of antibiotics in IBS. Most of these trials have utilised poorly absorbed antibiotics, in particular neomycin and rifaximin, presumably in an attempt to reduce their side effects by limiting their action to the gut microbiota only.

Neomycin, an aminoglycoside antibiotic, was one of the first antibiotics tested for the treatment of IBS. In one double-blind, randomised, placebo-controlled trial of 111 patients, neomycin resulted in a mean 35.0% improvement in IBS composite score, compared with a mean 11.4% improvement amongst those receiving placebo ($P < 0.05$).[116] When examining the proportion of participants achieving >=50% reduction in a composite IBS symptom score, there also was a significant effect in favour of neomycin (relative risk (RR) = 0.73; 95% CI 0.56 to 0.96). However, the use of neomycin is limited because of the risk of systemic adverse

effects, in particular ototoxicity in a small number of individuals. In another RCT, efficacy of norfloxacin was studied in 80 patients with IBS. Of the 40 participants who received norfloxacin, 15 (37.5%) responded, compared with none of those on placebo (RR = 0.63; 95% CI 0.49 to 0.60).[117]

Several RCTs have also investigated the benefit of rifaximin in IBS. In two RCTs of identical design, TARGET 1 and TARGET 2, 1260 patients with non-constipated Rome II-defined IBS were assigned to either rifaximin 550mg or placebo three times per day for 2 weeks.[44] In the two studies combined, participants in the rifaximin group, compared with those in the placebo group, were significantly more likely to have adequate relief of global IBS symptoms (40.7% vs. 31.7%, $P < 0.001$) and IBS-related bloating (40.2% vs. 30.3%, $P < 0.001$) for at least 2 of the first 4 weeks post-treatment. Interestingly, these improvements were maintained for at least 12 weeks post-treatment, the duration of follow-up in these RCTs. Lembo *et al.* reported similar results when 388 Rome-II defined IBS-D patients were randomised to either rifaximin 550mg or placebo twice daily for 2 weeks, with a significantly higher proportion of those in the rifaximin group reporting adequate relief of global IBS symptoms (52.0% vs. 44.0%, $P = 0.03$) and IBS-related bloating (46.0% vs. 40.0%, $P = 0.04$).[118] Other smaller RCTs have also examined the efficacy of rifaximin,[119, 120] and in a meta-analysis of five trials, which included 1805 non-constipated patients with IBS, there was a statistically significant benefit in favour of rifaximin (RR = 0.84; 95% CI 0.79 to 0.90) (Figure 2).[121] In addition, side effects appear to be no more common with antibiotics than with placebo (RR = 1.01; 95% CI 0.50 to 2.02).[122]

Even though rifaximin demonstrated an overall benefit over placebo over the short-term, an incremental reduction in the proportion of individuals with adequate relief of IBS symptoms was noted in both groups,[44] and demonstrating efficacy of a 2-week course of a drug over 12 weeks in a condition that, for many patients, is chronic and relapsing is

questionable. Due to a lack of clarity concerning the safety and efficacy of repeated courses of rifaximin in patients whose symptoms relapsed, Food and Drug Administration (FDA) approval for the use of rifaximin in IBS was not forthcoming after the TARGET 1 and 2 trials. TARGET 3, therefore, evaluated the safety and efficacy of repeated courses of rifaximin in responders.[45] The investigators randomised patients with IBS-D who responded to rifaximin during an open-label treatment phase to up to two further courses of rifaximin or placebo when IBS symptom relapse occurred. Among these 1074 participants who initially responded to open-label rifaximin, 382 (35.6%) did not experience symptom relapse for up to 18 weeks of follow-up. Among the 692 patients who relapsed, 636 were randomised to repeat treatment with either rifaximin or placebo. Participants who received repeat treatment with rifaximin, compared with those receiving placebo, were significantly more likely to meet the primary endpoint of an improvement in both abdominal pain and stool consistency for 2 weeks or more in the first 4 weeks after repeated treatment (38.1% vs. 31.5%, $P = 0.03$). Even in this trial with longer follow-up and repeated doses of rifaximin, adverse event rates were low and similar in both the rifaximin and placebo arms. The most common side effect was headache. One patient developed *Clostridium difficile* infection after 37 days of repeat rifaximin. However, the authors report that the patient had a history of *Clostridium difficile* infection and had completed a course of a cephalosporin antibiotic immediately before developing *Clostridium difficile* colitis.

The main hypothesis for the beneficial effect of rifaximin in IBS is its alteration of the composition of the gut microbiota. One study demonstrated that response to rifaximin in patients with IBS-D could be predicted by the results of lactulose breath testing measuring hydrogen and methane levels, which implies that rifaximin's mechanism of action may be via an alternation in the gut microbiome.[123] However, a number of other mechanisms such as modulation of the host inflammatory response, changes in the function of the microbiome by

affecting bacterial mucosal adherence, metabolism of bacterial end products, or bacterial virulence may be involved.[124] In addition, as with most drugs for IBS, approximately 60% of patients do not respond to rifaximin, suggesting that there may be other pathophysiological cause(s) for their IBS. The FDA have now licensed the use of rifaximin in IBS-D in the USA, but the drug remains unavailable in many countries for this indication and its efficacy, in terms of the therapeutic gain over placebo, remains modest and similar across all of the TARGET trials.

PROBIOTICS FOR IRRITABLE BOWEL SYNDROME

In the last decade, there has been a huge scientific and commercial interest in probiotics, not just for IBS or other gastrointestinal diseases, but also for other medical conditions or general well-being. Consumers, patients, and clinicians are often perplexed about the number of products available claiming to contain probiotics, of which many do not necessarily meet the strict criteria for being a probiotic. The Food and Agriculture Organisation of the United Nations and the World Health Organisation have defined probiotics as "live microorganisms, that when administered in adequate amounts, confer a health benefit on the host."[125] In addition, the International Scientific Association for Probiotics and Prebiotics have set the standard definitions for other products containing, or related to, probiotics.[126]

With the microbiome being proposed as a potential therapeutic target in IBS, there have been several attempts to modify its composition using probiotics. However, despite several in vitro, animal models, and human trials, the exact mechanism(s) of action of most probiotics remain incompletely understood.[127] Probiotics, such as *Lactobacillus acidophilus* NCFM, have been shown to be able to modify pain receptors in the mouse and human gut, an effect that may improve visceral hypersensitivity in those with IBS.[128, 129] Others have demonstrated normalisation of interleukin levels in patients with IBS following treatment with *Bifidobacterium longum* 35624, suggesting an immunomodulatory role of some probiotics.[130] Finally, probiotics may also modulate the central nervous system as demonstrated by a reduction in both depression scores and brain activation to fearful stimuli, using functional magnetic resolution imaging, following treatment with *Bifidobacterium longum* NCC3001.[131]

There have been numerous meta-analyses examining the efficacy of probiotics in IBS. One of the most recent identified 37 RCTs that investigated the efficacy of probiotics compared with placebo in 4403 patients using dichotomous outcome variables to assess the persistence of IBS symptoms.[121] Of these RCTs, 21 (n = 1931) investigated combination probiotics, eight (n = 893) *Lactobacillus*, three (n = 528) *Bifidobacterium*, two (n = 579) *Saccharomyces*, two (n = 418) *Escherichia*, and one (n = 54) *Streptococcus*. There was a significant effect in favour of combination probiotics (RR = 0.79; 95% CI 0.68 – 0.91), *Escherichia* (RR = 0.86; 95% CI 0.79 to 0.93), and *Streptococcus* (RR = 0.72; 95% CI 0.53 to 0.99) (Figure 3). There was no benefit detected over placebo for *Lactobacillus* (RR = 0.82; 95% CI 0.63 to 1.06) when all eight studies were analysed but when three studies including only *Lactobacillus plantarum DSM 9843* (n = 314), a benefit of this specific strain was observed (RR = 0.67; 95% CI 0.51 to 0.87). There was no benefit over placebo for *Bifidobacterium* (RR = 0.70; 95% CI 0.48 – 1.01), or *Saccharomyces* (RR = 0.92; 95% CI 0.82 – 1.03). The meta-analysis also reported data for each group according to global or abdominal pain symptom scores, and bloating scores (Table 1). There were 19 trials, evaluating 1341 patients, using combination probiotics which reported a statistically significant improvement in global or abdominal pain symptom scores (standardised mean difference (SMD) = -0.31; 95% CI -0.44 to -0.17) with active treatment compared with placebo. There was a trend towards a benefit for *Bidifobacterium* species on global or abdominal pain scores (SMD = -0.18; 95% CI -0.92 to 0, $P = 0.05$), and for combination probiotics on bloating scores (SMD = -0.15; 95% CI -0.31 to 0.01, $P = 0.06$) but no difference in the other analyses. Finally, there was no significant difference in terms of adverse events (RR = 1.09; 95% CI 0.91 to 1.29).

Since the publication of this meta-analysis, there have been numerous other RCTs of different probiotic strains, species, or combinations of probiotics. *Andresen et al.* investigated

the efficacy and safety of heat inactivated *Bifidobacterium bifidum MIMBb75* in a placebo-controlled trial recruiting 443 patients with Rome III IBS. The probiotic group were more likely to meet the composite endpoint of at least 30% improvement of abdominal pain and adequate relief of overall IBS symptoms (RR = 1.7; 95% CI 1.3 to 2.4).[132] Bai *et al.* not only assessed the efficacy of a combination probiotic with four strains of *Bifidobacterium* in a recently published RCT, but also performed 16S rRNA gene sequencing as well as microbial diversity analysis and quantified faecal metabolites from stool samples.[133] The proportion of responders, using a composite endpoint of improvement in both abdominal pain and stool consistency, in the probiotic group was significantly higher than in the placebo group (67.6% vs. 36.6%, $P < 0.001$). Although there was no significant difference in the diversity of gut microbiota, participants who received the probiotics had significantly higher abundance of bacteria producing SCFAs and higher concentration of SCFAs, including butyrate, valerate, and caproate in their stool samples. In a recent phase II RCT recruiting 366 patients with Rome IV-defined IBS, Quigley *et al.* reported that patients who received MRx12234, a live biotherapeutic product containing a strain of *Blautia hydrogenotrophica*, had numerically higher response rates (24.1% vs. 17.5%, $P = 0.063$) and improved abdominal pain scores (38.2% vs. 31.7%, $P = 0.098$), compared with those who received placebo, although this was not statistically significant.[134] There was no increased rate of adverse events among those receiving active medication compared with placebo, but larger studies with greater power are required to investigate MRx12234.

All these trials suggest that some probiotics are efficacious in at least some patients with IBS. However, because of a lack of comparative data, it is still unknown whether there is a particular strain or species that is more efficacious in IBS, and whether a particular predominant symptom, or IBS subtype is more likely to be improved by their use. Other uncertainties, such as the optimal dose, strain or combination of probiotics, or the optimal

treatment duration, also exist partly because of the lack of high-quality trials. Heterogeneity amongst trial results in almost all meta-analyses, presumably resulting from differences in study protocols, populations of patients studied, strains or species of probiotic used, durations of treatment, and outcome measures, remain a challenge for those trying to understand the current literature. In addition, many trials do not conform to the recommendations for the design of treatment trials for disorders of gut-brain interaction,[135] in terms of their duration, nor do they use FDA or European Medicines Agency recommended endpoints to assess efficacy for IBS. This means that they have not been assessed as stringently as most available licensed drugs for the treatment of IBS. Reassuringly, a framework for trials using probiotics in IBS has been proposed recently and may help investigators designing future trials.[136]

FUTURE CHALLENGES

The first major challenge is to define IBS. A purely symptom-based criteria reliant on gastrointestinal symptoms alone leads to a heterogeneous group of individuals being recruited into clinical trials. This in turn leads to the modest efficacy of most medications for IBS observed in RCTs, as well as high placebo response rates. There are also several challenges in gut microbiome research. The discovery of *Helicobacter pylori* and its role in the pathophysiology of peptic ulcer disease 40 years ago represents, arguably, the most successful story in the field of gut microbiome.[137, 138]. Despite the advances in laboratory techniques in the last two decades, our understanding of the microbiome is still limited. In order to establish the role of the gut microbiome in any disease state, we need to define a healthy microbiome. We need to understand not only its composition but also the complex interactions among the microorganisms and between the microbiota and their host. With so many factors affecting the microbiome, there exists a large microbiome variance among healthy individuals, especially at the level of species and strains of microorganisms.[56, 139, 140, 141] Variability does not only exist among only healthy individuals, or within the same individual over time, but also occurs in different areas of the gut such as the stomach, small bowel, or colon,[96] and as previously discussed, in the gastrointestinal lumen compared with the mucosa.[96, 97, 98, 99] These issues make it even more challenging to untangle the composition and function of the gut microbiome in health and disease. Finally, we need to accurately identify which changes in the microbiome, be it the bacterial composition, function, interaction among themselves or with their host, are responsible for IBS and then reverse these changes to improve or cure symptoms of IBS.

CONFLICTS OF INTEREST

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Table 1. Efficacy of probiotics in the treatment of IBS from a meta-analysis of RCTs according to global symptom or abdominal pain scores and bloating scores.[121]

Subgroups	Number of RCTs	Total number of patients	Standard Mean Difference (95% CI)	P value
I. Global symptom or abdominal pain scores				
1. Combination probiotics	19	1341	-0.31 (-0.44 to -0.17)	<0.001
2. *Lactobacillus*	8	989	-0.09 (-0.25 to 0.06)	0.23
3. *Saccharomyces*	4	388	0.12 (-0.27 to 0.50)	0.55
4. *Bifidobacterium*	3	501	-0.46 (-0.92 to -0.00)	0.05
II. Bloating scores				
1. Combination probiotics	17	1155	-0.15 (-0.31 to 0.01)	0.06
2. *Lactobacillus*	2	537	-0.00 (-0.17 to 0.17)	0.99
3. Saccharomyces	3	209	-0.01 (-0.36 to 0.34)	0.95
4. Bifidobacterium	3	501	-0.30 (-0.68 to 0.09)	0.13

RCT = randomised controlled trial

Figure 1. Factors affecting the gut microbiome.

Figure 2. Gut microbiome and dysbiosis

Figure 3. Forest plot of randomised controlled trials of rifaximin vs. placebo in irritable bowel syndrome: effect on persistence of symptoms.[121]

Figure 4. Forest plot of randomised controlled trials of probiotics vs. placebo in irritable bowel syndrome: effect on persistence of symptoms.[121]