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# Intestinal microbiota in functional bowel disorders: a Rome foundation report

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## ABSTRACT

It is increasingly perceived that gut host–microbial interactions are important elements in the pathogenesis of functional gastrointestinal disorders (FGID). The most convincing evidence to date is the finding that functional dyspepsia and irritable bowel syndrome (IBS) may develop in predisposed individuals following a bout of infectious gastroenteritis. There has been a great deal of interest in the potential clinical and therapeutic implications of small intestinal bacterial overgrowth in IBS. However, this theory has generated much debate because the evidence is largely based on breath tests which have not been validated. The introduction of culture-independent molecular techniques provides a major advancement in our understanding of the microbial community in FGID. Results from 16S rRNA-based microbiota profiling approaches demonstrate both quantitative and qualitative changes of mucosal and faecal gut microbiota, particularly in IBS. Investigators are also starting to measure host–microbial interactions in IBS. The current working hypothesis is that abnormal microbiota activate mucosal innate immune responses which increase epithelial permeability, activate nociceptive sensory pathways and dysregulate the enteric nervous system. While we await important insights in this field, the microbiota is already a therapeutic target. Existing controlled trials of dietary manipulation, prebiotics, probiotics, synbiotics and non-absorbable antibiotics are promising, although most are limited by suboptimal design and small sample size. In this article, the authors provide a critical review of current hypotheses regarding the pathogenetic involvement of microbiota in FGID and evaluate the results of microbiota-directed interventions. The authors also provide clinical guidance on modulation of gut microbiota in IBS.

## INTRODUCTION

Functional gastrointestinal disorders (FGIDs) are defined by symptom-based diagnostic criteria that combine chronic or recurrent symptoms attributable to the GI tract in the absence of other pathologically-based disorders.<sup>1</sup> The FGIDs are classified into six major categories for adults: oesophageal, gastroduodenal, bowel, functional abdominal pain syndrome, biliary and anorectal. Of these, the

functional bowel disorders (FBD) constitute one of the most common reasons for seeking healthcare,<sup>2</sup> and they are associated with poor health-related quality of life<sup>3–5</sup> and substantial costs to society.<sup>6–9</sup> The pathophysiological mechanisms underlying these disorders are incompletely known, but abnormal gastrointestinal (GI) motility, visceral hypersensitivity, altered brain–gut function, low-grade inflammation, psychosocial disturbance and intestinal microbes may contribute.<sup>10–12</sup>

The human body is inhabited by a complex community of microbes, collectively referred to as microbiota.<sup>13</sup> It is estimated that the human microbiota contains  $10^{14}$  cells, which outnumber the human cells in our bodies by a factor of ten.<sup>14</sup> A vast majority of these are found in the GI tract, with a continuum from  $10^1$ – $10^3$  bacteria per gram of content in the stomach and duodenum to  $10^{11}$ – $10^{12}$  cells per gram in the colon.<sup>15</sup> Moreover, the microbial composition differs between these sites,<sup>16</sup> and there are also significant differences between the microbiota present in the gut lumen and the microbiota attached to and embedded in the mucus layer of the GI tract.<sup>17</sup> The microbiota is taxonomically classified via the traditional biological nomenclature (phylum–class–order–family–genus–species) and currently more than 50 bacterial phyla have been described, of which 10 inhabit the colon and three predominate: the Firmicutes, Bacteroidetes and the Actinobacteria; other sites display a different microbial composition.<sup>18–19</sup> A challenge for researchers and clinicians is that most of the microbial diversity in the human GI tract is not currently represented by available cultured species,<sup>20</sup> but during recent years, the use of culture-independent techniques to study the gut microbiota has increased the understanding of the role of gut microbiota in health and disease.<sup>14</sup>

Several lines of evidence indicate that bacteria may be involved in the pathogenesis and pathophysiology of FBD, through the metabolic capacity of the luminal microbiota, and the potential of the mucosa-associated microbiota to influence the host via immune–microbial interactions.<sup>21</sup> For instance, many subjects with irritable bowel syndrome (IBS) report symptom onset following an enteric infection.<sup>22</sup> There are also studies reporting positive effects of treatments directed at gut microbiota in patients with FBD.<sup>23–24</sup> Moreover, small intestinal

bacterial overgrowth (SIBO)<sup>25</sup> and altered intestinal microbiota<sup>26</sup> are implicated in subgroups of FBD patients. However, the clinical relevance of these findings remains unclear and, therefore, we sought to critically review the existing literature on the role of intestinal microbiota in FBD, focusing predominantly on IBS, and to provide recommendations for how to implement the current knowledge into clinical practice and to guide future research.

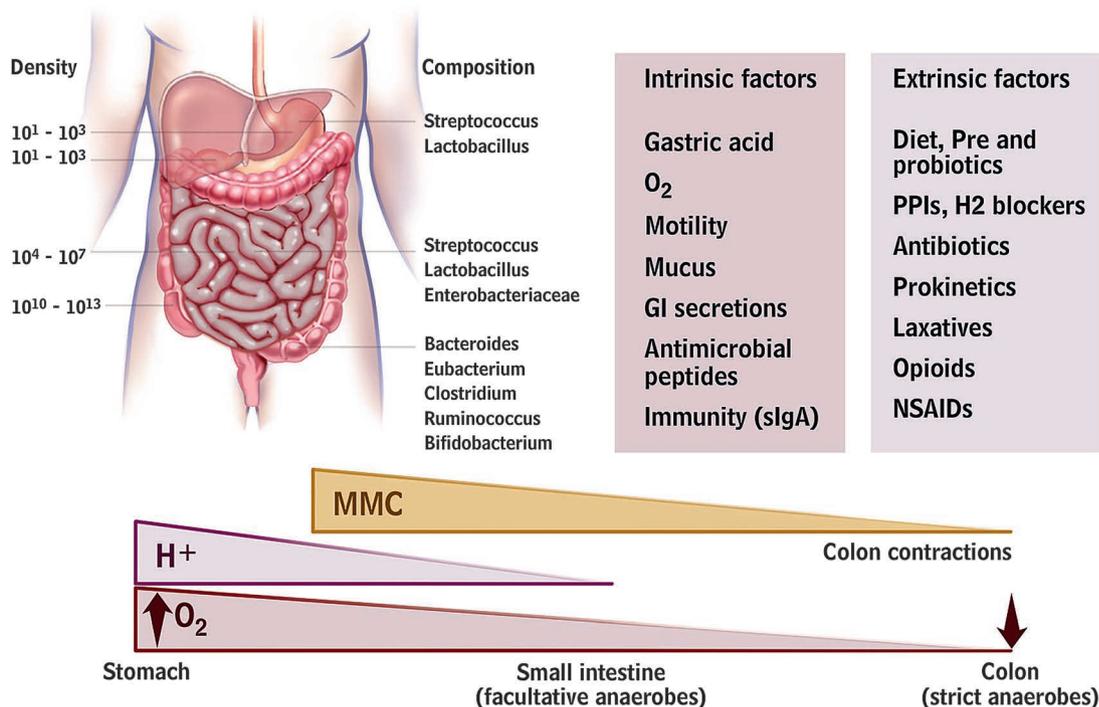
This manuscript is a synthesis of the endeavour of the Rome Foundation Committee Report. More in-depth description of the work produced by this team is provided as online supplementary material.

### CURRENT KNOWLEDGE OF THE MICROBIOTA

A relationship, often termed symbiosis, has developed between the host and the intestinal microbiota over millions of years. Host genetic and immune as well as environmental factors influence intestinal microbiota composition which in turn shape host immunity and physiology within and beyond the gut (figure 1). Recent human studies demonstrate a hitherto unimagined complexity of the human gut microbiota with hundreds of phylotypes, of which 80% remain uncultured.<sup>19</sup> Of the 10 bacterial phyla detected in the gut the Firmicutes, Bacteroidetes and Actinobacteria predominate, of which the Firmicutes is the most dominant and diverse phylum in the GI tract.

Facultative anaerobes account for <0.1% of the total bacteria detected in faecal samples. A recent paper suggested that the human GI tract microbiota can be divided into three robust clusters called enterotypes formed by groups of species that jointly contribute to their respective preferred community composition.<sup>27</sup> Remarkably, these enterotypes do not vary by patient characteristics, such as nation, gender, age or body mass index, although these findings are based on relatively small numbers of subjects. While most studies used faecal material, this does differ somewhat from the bacteria adherent to the mucosa, which are likely to interact most strongly with the host.<sup>28</sup>

Babies are born with sterile intestines but are rapidly colonised by bacteria from their immediate environment, most importantly their mother's vagina and gut.<sup>29</sup> Early colonisers of the neonatal gut are mainly aerobes (such as staphylococci, streptococci and enterobacteria), while late colonisers are strict anaerobes (such as eubacteria and clostridia) as the total microbiota become more complex, more stable and converge to a common pattern.<sup>30,31</sup> The microbiota continue to evolve until adulthood with a gradual increase in *Bacteroides* spp., a decline in *Lactobacillus* spp. after the age of five and a decline in *Bifidobacterium* spp. in late teenage.<sup>32</sup> Changes also occur in extreme old age when *Bacteroides* spp. decrease while *Enterococcus* spp. and *Escherichia coli* increase.<sup>33,34</sup> Industrialisation has



**Figure 1** Gut microbiota and the intrinsic and extrinsic factors that can affect its distribution and composition. A number of host mechanisms participate in gut microbiota modulation, including gastric acid secretion, fluid, anticomensal slgA and antimicrobial peptide production, and gastrointestinal (GI) motility. Drugs that block acid secretion and affect GI motility can indirectly alter the microbiota. Antibiotics, depending on spectrum and dosage, will directly affect microbiota composition. Dietary modifications, including probiotic and fibre supplements, will also affect microbiota composition. MMC, migrating motor complexes; H<sup>+</sup> hydrogen ions; O<sub>2</sub>, partial oxygen tension; slgA, secretory immunoglobulin A; PPI, proton pump inhibitor; NSAID, non-steroidal anti-inflammatory drug.

changed both our diet and microbiota as evidenced by comparing the faecal microbiota of African rural children with a polysaccharide-rich diet with Italian city children on a high fat, high protein diet. African children have a significant enrichment in Bacteroidetes, especially *Prevotella* and *Xylanibacter* genera known to contain genes for xylan hydrolysis<sup>35</sup> (figure 2). Whole grain cereals,<sup>36</sup> resistant starch<sup>37 38</sup> and low residue diets profoundly alter the microbiota.<sup>39</sup> Although there is evidence indicating that obese individuals have an increase in Firmicutes and a decrease in Bacteroidetes (a difference likely related in part to different diets<sup>40</sup>), other studies failed to support these observations.<sup>41 42</sup> Many dietary prebiotics including oligofructose,<sup>43</sup> lactulose,<sup>44 45</sup> lupin kernel,<sup>46</sup> inulin-containing juices<sup>47</sup> and arabinoxylan-oligosaccharides<sup>48</sup> significantly alter human faecal microbiota. The concept of poorly absorbed but fermentable oligo-, di- and monosaccharides and polyols (FODMAPs) includes many substances which are substrates for bacterial metabolism and may therefore alter the microbiota but this has as yet not been studied.

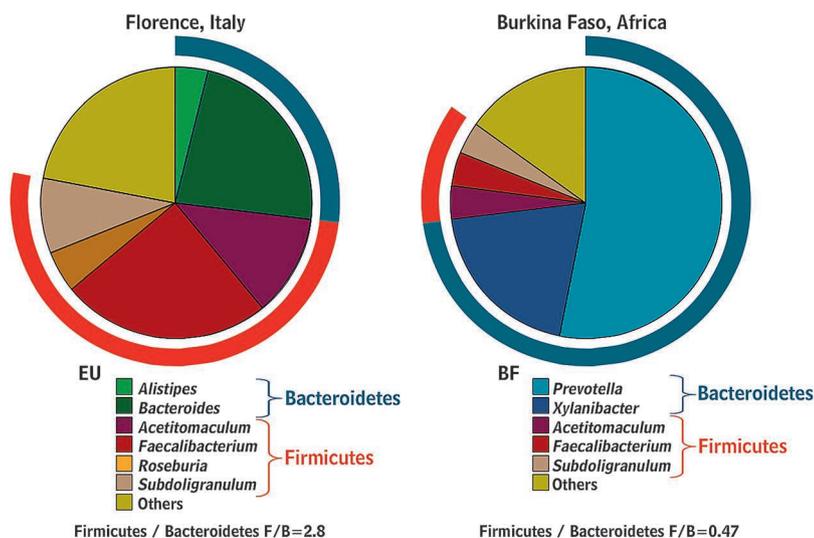
Most high fibre diets alter the microbiota and accelerate transit. Accelerating transit using senna increased the production of short chain fatty acids (SCFAs) but reduced faecal methanogens, the opposite to the effect of loperamide.<sup>49</sup> Accelerating transit with cisapride also increases production of SCFAs, particularly propionic and butyric acids.<sup>50</sup> Acetate, which predominates in the colonic contents, is largely inhibitory. In contrast, propionate and butyrate stimulate motility, activate propulsive ileal motor patterns in humans<sup>51</sup> and ensure that bacteria are propelled from the ileum to the colon. The normal microbiota also strongly influence the mucosal immune system<sup>52 53</sup> which is underdeveloped in germ-free animals, who have reduced T cells, immunoglobulin A producing B cells and intraepithelial T cells.<sup>52 54–56</sup> Twin studies suggest that the host genotype influences the gut

microbiota, although results remain conflicting because of the inability to control for shared environmental factors.<sup>40 57</sup> One of the most important genetic effects is mediated via the innate immune response. Thus, mice lacking the bacterial sensing receptor nucleotide-binding oligomerisation domain-containing protein-2 showed significantly more Bacteroidetes as well as Firmicutes compared with wild-type mice.<sup>58</sup>

Modulation of the microbiota induces visceral hypersensitivity in mice, which is reduced by *Lactobacillus paracasei* NCC2461 secreted products.<sup>59</sup> *Lactobacillus acidophilus* NCFM and *Lactobacillus paracasei* NCC2461 also modulate visceral pain perception in rodents.<sup>60 61</sup> Transient perturbation of the microbiota with antimicrobials alters brain-derived neurotrophic factor expression, exploratory behaviour and colonisation of germ-free mice suggesting that intestinal microbiota impact is not limited to the gut and the immune system, but may involve the central nervous system.<sup>62</sup> (Note: this last sentence appears run-on but I can't quite decipher how to fix it.)

#### APPROACHES TO THE STUDY OF MICROBIOTA

Approaches to the study of microbiota and relative advantages/pitfalls are reported in box 1. Culture-based studies reveal that the gut microbiota is a highly complex community (box 1).<sup>63</sup> Although culturing remains valuable for identifying functional groups and for selective enumeration (eg, of pathogens), new culture-independent approaches provide more powerful and convenient methodologies for monitoring changes in the GI tract community (table 1). Information on the diversity of microbes that colonise the gut has expanded rapidly over the past 15 years, based largely on the analysis of the small subunit ribosomal RNA (16S rRNA for Bacteria and Archaea, 18S rRNA for Eukaryotes) gene sequences that can be obtained by direct amplification from nucleic acids extracted from gut or stool samples.<sup>64</sup> This information provides the basis for a range of complementary techniques for enumerating gut bacteria, including fingerprinting methods such as denaturing gradient gel electrophoresis<sup>65</sup> and targeted methods such as fluorescent in situ hybridisation and quantitative PCR. The arrival of new high-throughput sequencing approaches and 16S rRNA-based microarraying has further accelerated the supply of data by allowing amplified 16S rRNA sequences to be analysed in depth without the need for 'classical' cloning and sequencing methods.<sup>66 67</sup> Although culturing may bias against bacteria that are hard to grow in the laboratory, PCR amplification biases against certain groups of gut bacteria. For example, bifidobacterial 16S rRNA sequences are often under-represented among amplified products, although more reliably enumerated by 16S rRNA-targeted fluorescent in situ hybridisation detection or quantitative PCR.<sup>37</sup> While most molecular enumeration methods target 16S rRNA, some are based on more functionally relevant genes, for



**Figure 2** Gut microbiota composition in African children living in rural areas with a polysaccharide-rich diet when compared with Italian city children.<sup>35</sup> (Reprinted with permission from Proc Natl Acad Sci USA).

## Box 1 Approaches to the study of intestinal microbiota

- ▶ Breath tests are not validated to accurately detect small intestinal bacterial overgrowth.
- ▶ Rapid molecular approaches have largely replaced cultural approaches for enumeration of the dominant gastrointestinal (GI) tract microbiota.
- ▶ Cultural microbiology remains crucial for investigating microbial diversity and for the selective isolation of representatives of key functional groups, including pathogens.
- ▶ Culture-independent approaches to study the GI tract microbiota can answer the questions:
  - Which microbes are present in the GI tract? (16S rRNA gene-based approaches)
  - What microbial genes are present in the GI tract? (metagenomics)
  - What are GI tract microbes doing? (metatranscriptomics, metaproteomics, metabonomics/metabolomics).
- ▶ The possibilities of using high-throughput approaches and their depth of analysis are increasing rapidly, but it is important they are applied with careful reference to well-defined scientific questions.

example, involved in methanogenesis or butyrate synthesis.

High-throughput DNA sequencing provides completely new possibilities for ‘-omics’-based analyses of the gut microbiota.<sup>19</sup> Draft genomes of cultured gut bacteria can now be produced rapidly and at little cost.<sup>68</sup> In addition, these methods can be applied to DNA recovered from gut or stool samples, and the analysis of the resulting complex mixture of sequences is referred to as metagenomics.<sup>69–70</sup> The ability to analyse multiple gene sequences from large numbers of samples, complemented with functional screening and characterisation of randomly cloned DNA fragments from the GI tract, is currently being exploited to uncover changes in disease states including in inflammatory bowel disease (IBD). A related technology, metatranscriptomics, uses high-throughput sequencing or microarray analysis to examine RNA expressed in GI tract samples, thus focusing on bacteria that are transcriptionally active. Another potentially powerful tool, metaproteomics, employs protein separation and sequencing techniques to describe the major proteins present in gut or stool samples.<sup>71–72</sup> These ‘meta-omics’ approaches rely in primary sequencing and annotation data.<sup>73–74</sup> Thus, they rely heavily on the availability of genome sequences and functional information from cultured reference bacteria, which means there are considerable benefits from combining different approaches to gut microbiota analysis. A final ‘omics’ approach, metabonomics, is not linked directly to genetic information of the microbes, but examines the metabolite profiles resulting from total microbial activity in the gut. Since many of these metabolites exert biological effects (some positive, some negative) on the host, such analysis can provide a direct measure of the consequences of microbial activity in the gut, although excluding cell-mediated effects and direct identification to a target microbial species.

Breath testing has been used to detect SIBO in IBS patients by non-invasively detecting hydrogen producing bacteria or methane producing archaea within the gut lumen. The breath test is based on the concept that hydrogen gases are produced by colonic bacterial fermentation in response to ingestion of a test sugar. They rapidly diffuse into the blood, are excreted by breath, and can be collected and quantified.<sup>75</sup> If SIBO exists, the timing of this fermentation would be altered but the criteria for abnormal tests lack validity (figure 3).

#### DIFFERENCES IN THE MICROBIOTA IN FBD AND THE LINK TO PATHOPHYSIOLOGY

There is little known about the small intestinal microbiota as the small intestine is relatively inaccessible (summarised in table 2; box 2).<sup>75–85</sup> Culture studies show considerably fewer bacteria compared with the colon with a marked gradient from duodenum to distal ileum. The bacteria are typically Gram-positive aerobes proximally and Gram-negative and Gram-positive anaerobes and facultative anaerobes in the terminal ileum. Culture-independent studies of the small intestinal microbiota are in their infancy but suggest complexity not appreciated by standard culture techniques, including marked individual differences, fluctuations over time (even within the same day), age-related differences and several phylotypes not previously identified.<sup>86–88</sup> Moreover, a recent paper indicated that the small intestinal microbiota are driven by a rapid uptake and conversion of available simple carbohydrates in which *Streptococcus* spp. play an important role.<sup>89</sup>

The role of SIBO in the pathogenesis of IBS is very controversial because the breath tests employed to establish this role have not been validated.<sup>90–91</sup> Even the validity of the ‘gold standard’, jejunal cultures  $>10^5$  cfu/ml with colonic-type bacteria, has been challenged, largely because this cut-off was established from samples following surgical diversion.<sup>91</sup> Studies in IBS patients showed relatively few bacteria in the duodenum and proximal jejunum and no obvious differences from controls (table 2). Preliminary studies suggest that more IBS patients have SIBO when a lower cut-off of  $>10^3$  cfu/ml is used but well-designed studies are needed.<sup>82–85</sup> Available molecular studies are not adequately designed to establish whether SIBO is involved in IBS but have significant potential.

Several confounding factors, including acid suppression by proton pump inhibitors (PPIs) and altered motility, have been implicated in the studies of SIBO and IBS.<sup>92–94</sup> Some studies suggest that PPI use might lead to symptomatic SIBO or at least increased numbers of bacteria and that following antibiotics they accelerate recurrence, but this depends on the tests employed and criteria applied.<sup>95</sup> Although the link between SIBO and IBS is largely based on breath testing, most positive lactulose breath tests reflect rapid transit to the caecum rather than true SIBO<sup>94</sup> (figure 4). Other factors such as antibiotics, probiotics and

**Table 1** Main features of culture-independent detection methods of gut microbiota

Question	Target	Approach	Data generated	Can microbes be identified directly?	Main benefit	Main limitation
Which microbes are present in the GI tract?	Isolates	Cultivation	Phenotypic characterisation	Yes	Accurate species identification	Not representative
	16S rRNA gene	Cloning and sanger sequencing	Phylogenetic identification	Yes	Complete 16S rRNA gene sequence data	Cloning bias
	16S rRNA gene	High-throughput sequencing	Phylogenetic identification	Yes	High-throughput data generation	Short reads
	16S rRNA gene	Fingerprinting	Community profile	No	Fast comparison between communities	No direct link with phylogeny
	16S rRNA	FISH	Cell numbers	Yes	Accurate enumeration	Dependent on 16S rRNA databases
What microbial genes are present in the GI tract?	16S rRNA gene	qPCR	16S rRNA gene abundances	Yes	Wide dynamic range	Dependent on 16S rRNA databases
	16S rRNA gene	Phylogenetic microarray	Phylogenetic identification	Yes	High-throughput phylogenetic profiling	Dependent on 16S rRNA databases
	Community DNA	Sequence-based metagenomics	Gene sequences	Not always	High-throughput data generation	Function mainly based on predictions
	Community DNA	Function-based metagenomics	Functional properties encoded on DNA fragment	Not always	Functional properties linked to DNA sequences	Suitable cloning host/system and screening assays needed
What are GI tract microbes doing?	mRNA	Metatranscriptomics	Community gene expression	Not always	Direct information about microbial activity	Community RNA extraction challenging
	Proteins	Metaproteomics	Community protein production	Not always	Direct information about microbial activity	No uniform protocol for all cell fractions
	Metabonomics	Metabonomics/metabolomics	Community metabolite profiles	No	Microbiota activity representation	No link with microbes or its function
	Lactulose hydrogen breath test	Measuring GI tract gas production	Hydrogen and methane breath content	No	Unclear, simple test but not validated for diagnosing SIBO	May simply measure small intestinal transit time to caecum
	Glucose hydrogen breath test	Measuring GI tract gas production	Hydrogen breath content	No	Same as above	Poor sensitivity; misses distal SIBO

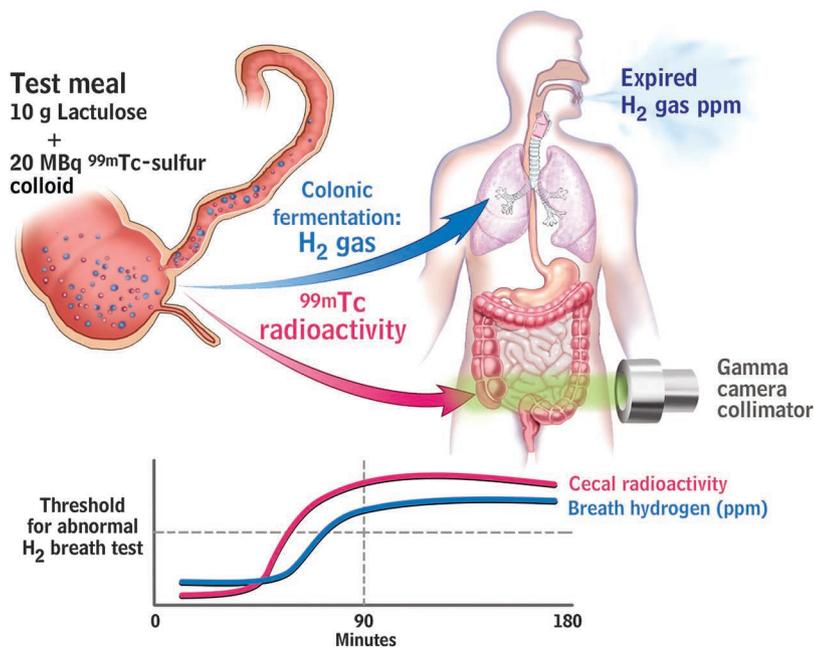
FISH, fluorescent in situ hybridisation; GI, gastrointestinal; qPCR, quantitative PCR; SIBO, small intestinal bacterial overgrowth.

prebiotics, and other dietary items such as FODMAPs could also influence microbiota in IBS patients and result in a potentially spurious association.

Earlier culture-based assessment of faecal microbiota obtained from patients with IBS demonstrated decreased faecal lactobacilli and bifidobacteria, and increased facultative bacteria dominated by streptococci and *Escherichia coli* as well as higher counts of anaerobic organisms such as *Clostridium*.<sup>96 97</sup> Studies using molecular-based techniques reveal changes in faecal microbiota composition in IBS versus controls (table 3). Interestingly, a recent study demonstrated that faecal microbiota of IBS patients could be grouped in a cluster which was completely different from that of healthy controls.<sup>114</sup> Nonetheless, results to date are inconsistent and sometimes contradictory (table 3). This may reflect differences in molecular techniques employed, the use of single samples that are not linked to fluctuating symptoms (especially as studies suggest IBS faecal microbiomes are less stable), and probably other factors such as diet and phenotypic characterisation of patients. In addition, it should be realised that faecal samples do not necessarily reflect other parts of the GI tract.

The finding that IBS can develop following infective gastroenteritis prompted studies evaluating the role of inflammation in IBS, but there are fewer studies that focus on the associated changes in gut microbiota, which might be just as significant. Infective gastroenteritis produces a profound depletion of the commensal microbiota,<sup>118</sup> whose production of metabolites such as SCFAs and antibiotics normally inhibits pathogen colonisation, as can be seen from the loss of colonisation resistance after antibiotics.<sup>119</sup> It is unclear just how completely and over what time span recovery occurs.

Infective gastroenteritis is common, with an incidence of 19/100 person years in the UK.<sup>120</sup> A third of episodes are viral (Norovirus/Rotavirus being the commonest). The commonest bacterial infections, *Campylobacter* and *Salmonella*, account for 10% and 3%, respectively. Onset of new IBS symptoms after a bout of infective gastroenteritis is relatively common, reported by 6%–17% of IBS patients,<sup>121</sup> while a recent internet survey reported 18%,<sup>122</sup> with around 40% beginning while travelling. The clinical features of post-infectious-IBS are predominantly those of IBS-diarrhoea (IBS-D).<sup>123 124</sup> A recent meta-analysis pooling 18 studies indicated a relative increased risk of developing IBS 1 year after bacterial gastroenteritis (mostly *Shigella*, *Campylobacter* and *Salmonella*), RR=6.5 CI (2.6–15.4), an effect still apparent at 36 months, RR=3.9 (3.0–5.0).<sup>125</sup> Viral gastroenteritis, in keeping with the lesser tissue injury, shows a reduced incidence of post-infectious-IBS compared with bacterial infections<sup>126 127</sup> in which the strongest risk factors are bacterial toxicity,<sup>128</sup> prolonged duration of diarrhoea,<sup>124</sup> rectal bleeding<sup>129</sup> and fever.<sup>125</sup> Acute enteritis is associated with a prolonged increase in mucosal cytotoxic T lymphocytes and increase in enteroendocrine



**Figure 3** The lactulose hydrogen breath test (LHBT) predominantly measures small intestinal transit rather than small intestinal bacterial overgrowth (SIBO) in irritable bowel syndrome (IBS) patients. Upper schematic shows ingestion of test meal with subsequent serial measurement of both H<sub>2</sub> gas, resulting from fermentation of the lactulose by intestinal bacteria, and Tc<sup>99</sup> scanning in the caecum. This latter measurement detects when the test meal has reached the caecum. The stylised drawing below shows a representative result from an IBS patient with serial measurements over time. The Tc<sup>99</sup> had already reached the caecum in large quantities before the H<sub>2</sub> PPM level has reached the threshold for an abnormal test. This demonstrates that the increased H<sub>2</sub> production results from fermentation by colonic bacteria, not by abnormal bacteria small intestine (ie, SIBO).<sup>94</sup>

cells.<sup>123</sup> Other studies have shown the importance of increased 5HT containing cells in IBS-D<sup>130</sup> and increased sensitivity in IBS-D with increased EC cell counts,<sup>131</sup> accelerated gut transit and visceral

hypersensitivity.<sup>132</sup> These effects on gut physiology will impact on the gut microbiota environment. An early study of children with acute gastroenteritis demonstrated alkalinisation of stool pH, likely due to the decrease in bacterial metabolites (SCFAs) and a fall in numbers of *Bacteroides*, *Bifidobacterium*, *Lactobacillus* and *Eubacterium*.<sup>133</sup> Conventional enumeration of faecal bacteria showed a 10-fold fall in anaerobes (*Bacteroidaceae* and *Eubacterium*), little change in aerobes, but 10<sup>9</sup> cfu/g of pathogens. Another study using conventional culture methods showed a reversal of the normal anaerobe/aerobe dominance during acute infection.<sup>134</sup> More recent human studies using modern culture-independent methods tended to confirm these findings.<sup>135</sup> 136 PCR-denaturing gradient gel electrophoresis profiling of 16S rRNA genes showed a reduced diversity, often associated with a dominant band suggesting overgrowth of one subtype, which may not always be the original pathogen. A recent clinical trial of an oral rehydration solution containing a prebiotic, amylase resistant starch in acute diarrhoea in India, including children aged 3 months to 5 years, used PCR primers directed at selected bacteria, for example, *Eubacterium* spp. and *Faecalibacterium prausnitzii*, key bacteria involved in starch fermentation. These studies showed a decline in some anaerobes (*Bacteroides* spp., *Eubacterium* spp. and *Faecalibacterium prausnitzii*) while other genera including *Bifidobacterium* spp. were unchanged.<sup>135</sup> This depletion of anaerobes could be due to acceleration of transit, which could lead to a loss of the anaerobic niche. Since these are the key bacteria involved in colonic salvage of unabsorbed carbohydrate,<sup>137</sup> this may also contribute to the diarrhoea phenotype by preventing fermentation to SCFAs, which are known to stimulate colonic salt and water absorption, both directly and by inducing

**Table 2** Summary of studies culturing small bowel microbiome

Study	Number of patients	Sample type	Microbiology results	Comments
Drasar and Shiner <sup>76</sup>	13 Diarrhoea, all investigations negative	Jejunal capsule	No difference from controls; no increased numbers of pathogens or non-pathogens	Possible IBS but not defined as IBS
Rumessen <i>et al</i> <sup>77</sup>	60 Patients suspected of SIBO	Proximal jejunal aspirate	15 With no predisposing cause had no evidence of SIBO; of 23 with SIBO, 4 had no predisposing cause	Groups poorly defined, 8 IBS identified and all negative for SIBO; 22 cases considered inconclusive
Corazza <i>et al</i> <sup>78</sup>	31 Chronic diarrhoea, no predisposing cause	Proximal jejunal aspirate	10 Had SIBO ( $\geq 10^6$ cfu/ml or colonic bacteria), 2 IBS, 8 other multiple other diagnoses	IBS not defined, and total IBS not clear
Bardhan <i>et al</i> <sup>79</sup>	10 Controls; 4 irritable colon; 22 other	Endoscopic aspirates from proximal jejunum	No positive cultures in irritable colon	Positive cultures in 11 cases, many postsurgical
Lewis <i>et al</i> <sup>80</sup>	23 With functional bowel disorders	Duodenal endoscopic aspirate	Mean control count $3.2 \times 10^2$ cfu/ml, no anaerobes, no sterile samples	No specific IBS, defined as functional bowel disorders
Sullivan <i>et al</i> <sup>81</sup>	7 IBS; 20 controls	Proximal jejunal biopsy using Watson capsule	No differences, flora similar to normal oropharyngeal flora	Colonic pathogen in 2 healthy subjects
Posserud <i>et al</i> <sup>82</sup>	162 IBS; 42 controls	Proximal jejunal aspirate	4% $\geq 10^5$ cfu, same as controls. Subanalysis using $\geq 5 \times 10^3$ , 43% IBS vs 12% controls	No correlation with motor pattern in IBS group
Kerckhoffs <i>et al</i> <sup>83</sup>	8 IBS; 9 controls	Proximal jejunal aspirate	No different number diagnosed with SIBO using multiple definitions	No differences also using molecular-based counts
Choung <i>et al</i> <sup>84</sup>	148 IBS; 542 'other indications to test for SIBO'	Duodenal endoscopic aspirate	2% IBS $> 10^5$ cfu/ml 10% in 'other' indications	Retrospective study 18% IBS $> 10^5$ cfu/ml
Pylaris <i>et al</i> <sup>85</sup>	85 IBS 150 non-IBS	Duodenal endoscopic aspirate	37% IBS $> 10^3$ cfu/ml 15.11% non-IBS	All investigated because of UGI bleed

IBS, irritable bowel syndrome; SIBO, small intestinal bacterial overgrowth; UGI, upper gastrointestinal.

## Box 2 Relevance of studies showing changes in microbiota in irritable bowel syndrome

- ▶ The relevance of small intestinal bacterial overgrowth in irritable bowel syndrome (IBS) remains unclear due to methodological problems, influence of confounding factors and large differences between studies.
- ▶ Heterogeneity of IBS and variation in methods used to study the faecal microbiota have resulted in conflicting reports of differences from healthy controls.
- ▶ The microbiome may contribute to IBS symptoms by altering gut neuromotor-sensory function, barrier function and/or the brain–gut axis.

increased expression of transporters.<sup>138–140</sup> Previous earlier studies in IBS-D suggest impaired SCFA concentrations and production rates in ex vivo incubation, which may also reflect reduced anaerobes.<sup>141</sup>

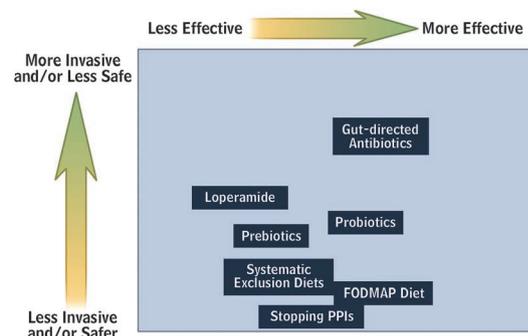
Another cause of depletion of anaerobes is broad-spectrum antibiotics. There are no RCTs, but epidemiological studies show an association between antibiotic use and an increased risk of PI-IBS.<sup>142</sup> A study of children showed that 3 months after *Salmonella* infection, vomiting, abdominal pain and diarrhoea were reported by 9.5% of those treated with antibiotics but only 2.9% of those who received no antibiotics.<sup>142</sup>

Changes in the interaction between intestinal microbiota and host factors (eg, age, diet, transit, host genetic factors, antibiotics) could be important for IBS pathophysiology. These factors, in turn, could be related to changes in homeostatic pathways including barrier function, neuromotor sensory function and the brain–gut axis.<sup>143–144</sup> For example, bidirectional signalling between the microbiota and the epithelium regulates epithelial secretion of mucus as well as other defence factors involved in regulating the microbiota. Changes in these factors (eg, changes in mucus layer and increased  $\beta$ -defensin-2 peptide) have been detected in patients with IBS and functional diarrhoea and suggest a microbiota–host immune system engagement.<sup>145–146</sup> In line with this concept, there is also recent demonstration that IBS patients have increased colonic mucosal expression of

receptors recognising specific microbiota-related substances (such as Toll-like receptor-4 which recognises bacterial lipopolysaccharides)<sup>147</sup> or increased titres of circulating antibodies against components of the indigenous microbiota (ie, anti-flagellin antibodies).<sup>148</sup> Several studies demonstrated low-grade activation of innate and adaptive mucosal immune response in large subgroups of patients with IBS.<sup>12–149</sup> Increased activated mast cells, CD3+ve, CD4+ve and CD8+ve T cells have been detected in both postinfectious IBS and non-specific IBS.<sup>12–149</sup> The relative importance of mast cells in this setting is demonstrated by the abundance of this immune cell type over other immunocytes and by increased release from mucosal biopsies of histamine, tryptase and prostaglandins.<sup>150–151</sup> Mast cells were located in closer vicinity to mucosal innervation and correlated with the severity and frequency of abdominal pain in patients with IBS.<sup>152</sup> There are potential implications of mucosal immune activation for sensorimotor dysfunction of patients with IBS. Histamine and tryptase released from mucosal biopsies of patients with IBS evoked increased mesenteric sensory afferent activation and induced visceral hypersensitivity via histamine-1 receptors and proteinase activated-2 receptors when applied to recipient rats.<sup>150–151</sup> Intestinal microbiota may well be an active participant in this scenario through stimulation of the immune system,<sup>153</sup> likely in the subgroup of subjects showing increased epithelial permeability which could<sup>154</sup> expose the immune system to an abnormal microbial antigenic load. Overall, the results suggest that bacterial–host interactions may be initiated by components of the microbiota that can cross the mucus and adhere to epithelial cells, inducing activation of the mucosal innate defence system even in the absence of mucosal destruction.

The use of probiotics, particularly in animal models, also demonstrates that their secreted products or metabolites can modulate contractility of intestinal smooth muscle and visceral sensitivity.<sup>59–61</sup> Moreover, application of probiotics can recover neuromotor-sensory dysfunction in IBS-like models.

Modulation of the brain–gut axis is particularly relevant in IBS because psychological comorbidity is common. Some forms of psychological stress in animal studies can induce shifts in the bacterial composition of the gut that is accompanied by systemic cytokine response and increased intestinal permeability.<sup>155</sup> The interplay may be bidirectional as suggested by animal studies showing that the microbiota can affect brain chemistry and behaviour.<sup>156</sup> Nonetheless, for the time, the potential relevance of brain–microbiota interactions have yet to be shown in humans in general and in FBD in particular.



**Figure 4** Plot chart of currently available strategies for modifying gut microbiota aiming to demonstrate the relationship between the effectiveness and invasiveness/safety of the proposed approach. FODMAP, fermentable oligo-, di- and mono-saccharides and polyols; PPI, proton pump inhibitor.

## GI DISORDERS MIMICKING AND OVERLAPPING WITH FBDS

Although celiac disease, IBD or diverticulitis can coexist with IBS, an 'IBS' diagnosis in the presence of an organic disease may be challenging.

**Table 3** Summary of culture and molecular studies of colonic microbiome

Study	Subject	Sample	Method	Patient group	Main finding	Country of study
Balsari <i>et al</i> <sup>96</sup>	IBS (n=20) Ctrls (n=20)	Faeces	Culture	IBS	↓ Coliform bacteria ↓ <i>Lactobacillus</i> spp. ↓ <i>Bifidobacterium</i> spp.	Italy
Si <i>et al</i> <sup>98</sup>	IBS (n=25) Ctrls (n=25)	Faeces	Culture	IBS	↓ <i>Bifidobacterium</i> ↑ Enterobacteriaceae ↓ <i>C. perfringens</i>	China
Mäilinen <i>et al</i> <sup>99</sup>	IBS (n=27) Ctrls (n=22)	Faeces	qPCR	IBS	↓ <i>B. catenulatum</i> ↓ <i>C. coccoides</i> group ↓ <i>Lactobacillus</i> spp.	Finland
Mättö <i>et al</i> <sup>100</sup>	IBS (n=26) Ctrls (n=25)	Faeces	Culture PCR-DGGE	IBS-D IBS-C	↑ <i>Veillonella</i> spp. ↑ <i>Lactobacillus</i> spp. ↑ Coliform bacteria	Finland
Maukonen <i>et al</i> <sup>101</sup>	IBS (n=24) Ctrls (n=16)	Faeces	PCR-DGGE Affinity capture	IBS	↑ Aerob to anaerob ratio ↓ Temporal stability	Finland
Kassinen <i>et al</i> <sup>102</sup>	IBS (n=24) Ctrls (n=23)	Faeces	GC-profiling + sequencing of 16S rRNA genes qPCR	IBS-C IBS	↓ Temporal stability ↓ <i>C. coccoides</i> group ↓ <i>Collinsella aerofaciens</i> ↓ <i>C. cocleatum</i> ↓ <i>Coprococcus eutactus</i>	Finland
Rajilic-Stojanovic <sup>103</sup>	IBS (n=20) Ctrls (n=20)	Faeces	Microarray	IBS	Subgroup-diff (D, C, M) Proteobacteria and specific Firmicutes ↑	Finland
Kerckhoffs <i>et al</i> <sup>104</sup>	IBS (n=41) Ctrls (n=26)	Faeces Duodenal mucosa	FISH qPCR	IBS	Other Firmicutes, Bacteroidetes and bifidobacteria ↓ ↓ <i>Bifidobacterium</i> spp.	The Netherlands
Krogius-Kurikka <i>et al</i> <sup>105</sup>	IBS-D (n=10) Ctrls (n=23)	Faeces	GC-profiling + sequencing of 16S rRNA genes	IBS-D	↓ <i>B. catenulatum</i> ↑ Proteobacteria ↑ Firmicutes	Finland
Lyra <i>et al</i> <sup>106</sup>	IBS (n=20) Ctrls (n=15)	Faeces	qPCR	IBS-D IBS-C IBS-A	↓ Actinobacteria ↓ Bacteroidetes ↑ <i>R. torques</i> 94% ↓ <i>C. thermosuccinogenes</i> 85% ↑ <i>R. bromii</i> -like ↓ <i>R. torques</i> 93% ↑ <i>C. thermosuccinogenes</i> 85%	Finland
Tana <i>et al</i> <sup>107</sup>	IBS (n=26) Ctrls (n=26)	Faeces	Culture qPCR	IBS	↑ <i>Veillonella</i> spp. ↑ <i>Lactobacillus</i> spp.	Japan
Codling <i>et al</i> <sup>108</sup>	IBS (n=41) Ctrls (n=33)	Faeces Colonic mucosa	PCR-DGGE	IBS	↑ Temporal stability No significant difference	Ireland
Carroll <i>et al</i> <sup>28</sup>	IBS-D (n=10) Ctrls (n=10)	Faeces	Culture qPCR	IBS-D	Faecal/mucosal ↓ Aerobic bacteria <i>Lactobacillus</i> spp.	USA
Noor <i>et al</i> <sup>109</sup>	IBS (n=11) Ctrls (n=22) UC (n=13)	Faeces	PCR-DGGE + sequencing of 16S rRNA genes	IBS	↓ Bacterial species ↑ Biodiversity ↑ Biological variability of predominant bacteria	UK
Mäilinen <i>et al</i> <sup>110</sup>	IBS (n=44)	Faeces	qPCR	IBS	<i>R. torques</i> 94% symptom severity Other phylotypes neg assoc.	Finland
Ponnusamy <i>et al</i> <sup>111</sup>	IBS (n=11) Ctrls (n=8)	Faeces	DGGE + PCR of 16S rRNA genes	IBS	↓ Alanine & pyroglutamic acid & phenolic compounds <i>S. aureus</i> (17%)	Korea
Rinttilä <i>et al</i> <sup>112</sup>	IBS (n=96) Ctrls (n=23)	Faeces	qPCR	IBS	↓ <i>S. aureus</i> (17%)	Finland
Saulhier <i>et al</i> <sup>113</sup>	IBS (n=22) Ctrls (n=22) (Children)	Faeces	16S Metagenomic sequencing and DNA microarray	IBS	↓ <i>γ</i> Proteobacteria Classified IBS subtypes using sets of discriminant bacterial species	USA
Rajilic-Stojanovic <i>et al</i> <sup>14</sup>	IBS (n=62) Ctrls (n=42)	Faeces	Phylogenetic 16S rRNA microarray and qPCR	IBS	↑ Proteobacteria and specific Firmicutes ↓ Other Firmicutes, Bacteroidetes and bifidobacteria	Finland

Table 3 continued

Study	Subject	Sample	Method	Patient group	Main finding	Country of study
Carroll <i>et al</i> <sup>115</sup>	IBS-D (n=16) Ctrls (n=21)	Faeces/Colonic mucosa	T-RFLP fingerprinting of 16S rRNA - PCR	IBS-D	Diminished microbial biodiversity in faecal samples	USA
Paikkes <i>et al</i> <sup>116</sup>	IBS-D (n=27) IBS-C (n=26) Ctrls (n=26)	Colonic mucosa	FISH Confocal microscopy	IBS	Expansion of mucosa-associated microbiota; mainly bacteroides and clostridia; association with IBS subgroups and symptoms	UK
Jeffery <i>et al</i> <sup>117</sup>	IBS (n=37) Ctrls (n=20)	Faeces	Pyrosequencing 16SrRNA		Clustering of IBS patients—normal-like versus abnormal microbiota composition (increased ratio of Firmicutes to Bacteroidetes); association with symptom profile	Sweden

n, number of randomised subjects.

B, Bifidobacterium; C, constipation; Cl, Clostridium; ctrls, controls; D, diarrhoea; DGGE, denaturing gradient gel electrophoresis; FISH, fluorescent in situ hybridisation; IBS, irritable bowel syndrome; L, Lactobacillus; qPCR, quantitative PCR; R, Ruminococcus; S, Staphylococcus; T-RFLP, terminal restriction fragment length polymorphism.

Gluten causes coeliac disease in genetically susceptible people and causes gut dysfunction in mice and can generate IBS symptoms in the absence of coeliac disease.<sup>157</sup> Some patients with IBS lack tissue transglutaminase antibodies or histological markers of coeliac disease yet still respond symptomatically to a gluten-free diet. This entity is termed 'non-coeliac gluten sensitivity' or 'gluten sensitive IBS'.<sup>157–159</sup> The underlying mechanisms in humans remain unclear. Mouse models indicate that gluten can induce activation of innate immunity, increased small intestinal permeability,<sup>160</sup> neuro-muscular dysfunction<sup>159</sup> and dysbiosis<sup>161</sup> in the absence of autoimmunity.

IBS-like symptoms are common in IBD patients in long-standing remission, or are frequently reported in patients before the diagnosis of IBD.<sup>162 163</sup> It is possible that IBS and IBD coexist with a higher than expected frequency, or may exist on a continuum, with IBS and IBD at different ends of the inflammatory spectrum. A study investigating IBS symptoms in IBD patients who were thought to be in clinical remission demonstrated high levels of calprotectin levels; this suggests that in most cases IBS symptoms are the result of undetected ongoing inflammation.<sup>164</sup> Underlying mechanistic links are lacking but it is tempting to raise the hypothesis that the intestinal microbiota may be a common factor in both diseases.<sup>165</sup> In fact, as with IBS (tables 2 and 3), faecal<sup>166–171</sup> and mucosal-associated dysbiosis<sup>167 172–178</sup> has been described IBD.

A high proportion of patients hospitalised with acute diverticulitis continue to have persistent symptoms that mimic IBS<sup>179</sup> despite the absence of complications.<sup>180</sup> Some uncontrolled studies claim benefit from antibiotics and/or mesalazine suggesting a role for the microbiota in this syndrome.<sup>181</sup>

### TREATMENT IMPLICATIONS: ANTIBIOTICS, PROBIOTICS, PREBIOTICS AND SYNBIOTICS

As the microbiota may be disturbed in functional GI disorders, a potential treatment approach is to try to correct dysbiosis either by the administration of an antibiotic or a preparation of 'beneficial' bacteria (box 3).

#### Antibiotics

Despite evidence that previous antibiotic use may be related to the development of IBS,<sup>182 183</sup> and the fact that antibiotic treatment may increase the development of long-term digestive symptoms after bacterial gastroenteritis,<sup>142</sup> poorly absorbable antibiotics might still have therapeutic potential in this condition.<sup>184</sup> Neomycin was the original choice<sup>184 185</sup> although interest is now focused on a non-absorbed derivative of rifampicin called rifaximin.<sup>186</sup>

There are three fully-published, double blind, placebo controlled trials of rifaximin in IBS<sup>187–189</sup> and the data suggest an improvement in symptoms, especially bloating and flatulence for approximately 10 weeks following treatment<sup>187 189</sup>

## Box 3 Modulation of intestinal microbiota in functional bowel disorders

- ▶ A short course of a non-absorbable antibiotic such as rifaximin has been shown to moderately improve the symptoms of irritable bowel syndrome (IBS), particularly bloating and flatulence. Improvement persists after the cessation of treatment but the exact duration of this effect remains uncertain.
- ▶ The majority of trials of probiotics in IBS show some degree of efficacy although some of the early studies were of very poor quality.
- ▶ Prebiotics and synbiotics should theoretically have the potential in treating functional gastrointestinal disorders but there are as yet no reliable data to support this view.

with a therapeutic advantage over placebo around 10%. The doses used in these and other studies vary between 600 and 2400 mg daily for 7–14 days<sup>190–195</sup> but there remain concerns about antibiotic resistance and possible *Clostridium difficile* infection although so far these issues have not appeared to be a problem.<sup>196–200</sup>

Thus, a short course of gut-specific antibiotics may have utility in some patients with IBS but we

need to know more about predictors of treatment responsiveness, antibiotic resistance, the efficacy and safety of re-treatment schedules as well as the optimal dosing regimen.<sup>201 202</sup>

**Probiotics**

Probiotics are live microorganisms which when administered in adequate amounts confer a health benefit on the host<sup>203</sup> with the most commonly used being the lactobacilli and bifidobacteria. Probiotics can be packaged in many formulations containing just one organism or a mixture and have a wide range of activities with evidence supporting an effect on at least some of the putative pathophysiological mechanisms implicated in IBS, such as visceral hypersensitivity,<sup>59 60 204 205</sup> GI dysmotility,<sup>206–210</sup> intestinal permeability,<sup>204 211 212</sup> the intestinal microbiota<sup>213 214</sup> and immune function<sup>215</sup> although these effects can differ considerably between one organism and another. Thus, just because one organism is beneficial, this does not mean that related organisms will behave similarly. For use in gastroenterology, it is important that a preparation contains sufficient quantities of

**Table 4** Placebo controlled clinical trials of single or mixed probiotic preparations in IBS

Organism	n	Outcome	Reference
Studies in adult patients			
<i>S faecium</i>	54	↓ Global score	Gade <i>et al</i> <sup>216</sup>
<i>Lactobacillus acidophilus</i>	18	↓ Global score	Halpern <i>et al</i> <sup>217</sup>
<i>Lactobacillus plantarum</i> 299V	60	↓ Flatulence	Nobaek <i>et al</i> <sup>218</sup>
<i>L plantarum</i> 299V	20	↓ Pain, 'all IBS symptoms'	Niedzielin <i>et al</i> <sup>219</sup>
<i>L plantarum</i> 299V	12	Negative	Sen <i>et al</i> <sup>220</sup>
<i>L plantarum</i> MF1298	16	Deterioration of symptoms	Ligaarden <i>et al</i> <sup>221</sup>
<i>L ramosus</i> GG	25	Negative	O'Sullivan <i>et al</i> <sup>240</sup>
<i>L reuterii</i> ATCC 55730	54	Negative	Niv <i>et al</i> <sup>223</sup>
<i>L salivarius</i> UCC4331	75	Negative	O'Mahony <i>et al</i> <sup>215</sup>
<i>Bifidobacterium infantis</i> 35624	75	↓ Pain and composite score	O'Mahony <i>et al</i> <sup>215</sup>
<i>B infantis</i> 35624	362	↓ Pain and composite score	Whorwell <i>et al</i> <sup>224</sup>
<i>Bifidobacterium lactis</i> DN-173-010	274	↓ Digestive discomfort	Guyonnet <i>et al</i> <sup>225</sup>
<i>B lactis</i> DN-173-010	34	↓ Maximum distension & pain	Agrawal <i>et al</i> <sup>208</sup>
<i>Bifidobacterium bifidum</i> MIMBb75	122	↓ Global score	Guglielmetti <i>et al</i> <sup>226</sup>
<i>Bacillus coagulans</i> GBI-30, 6086	52	↓ Bowel movements	Dolin <sup>222</sup>
<i>Escherichia coli</i> Nissle 1917	120	↑ Treatment satisfaction	Kruis <i>et al</i> <sup>227</sup>
VSL#3® (x8)*	25	↓ Bloating	Kim <i>et al</i> <sup>209</sup>
VSL#3® (x8)*	48	↓ Flatulence	Kim <i>et al</i> <sup>210</sup>
Medilac DS® (x2)*	40	↓ Pain	Kim <i>et al</i> <sup>228</sup>
Mixture (x4)*	103	↓ Global score	Kajander <i>et al</i> <sup>229</sup>
Mixture (x4)*	86	↓ Global score	Kajander <i>et al</i> <sup>214</sup>
LAB4 (x4)*	52	↓ Global score	Williams <i>et al</i> <sup>230</sup>
Mixture (x4)*	106	Negative	Drouault-Holowacz <i>et al</i> <sup>231</sup>
Mixture (x2)*	40	↓ Pain	Sinn <i>et al</i> <sup>232</sup>
ProSymbioFlor® (x2)*	297	↓ Global score	Enck <i>et al</i> <sup>233</sup>
Cultura® (x3)*	74	Negative	Simrén <i>et al</i> <sup>234</sup>
Cultura® (x3)*	52	Negative	Sondergaard <i>et al</i> <sup>235</sup>
Mixture (x4)*	70	↓ Pain	Hong <i>et al</i> <sup>236</sup>
Studies in paediatric patients			
<i>L ramosus</i> GG	50	↓ Abdominal distension	Bausserman and Michail <sup>237</sup>
<i>L ramosus</i> GG	104	↓ Pain	Gawronska <i>et al</i> <sup>238</sup>
<i>L ramosus</i> GG	141	↓ Pain	Francavilla <i>et al</i> <sup>212</sup>
VSL#3® (x8)*	59	↓ Global score	Guandalini <i>et al</i> <sup>239</sup>

\*Number of organisms in a mixture.

n, number of randomised subjects.

IBS, irritable bowel syndrome; L ramosus, *Lactobacillus ramosus*; L reuterii, *Lactobacillus reuterii*; L salivarius, *Lactobacillus salivarius*; S faecium, *Streptococcus faecium*.

microbes which need to be acid and enzyme resistant with good mucosal adherence also being an advantage.

Table 4 lists the results of the fully published placebo controlled probiotic trials to date.<sup>208–210 212 214–240</sup> Unfortunately, their designs vary considerably;<sup>241–244</sup> some of the older studies are of poor quality, and few attempt to define the mechanism of action or assess whether symptomatic improvement is accompanied by a change in the microbiota. A recent systematic review reported that studies of poorer quality tended to show larger effects and published data indicate a publication bias, with non-reporting of negative effects in small trials.<sup>244</sup> Around three-quarters of these studies were positive, of which four were in children, although different symptoms improved and the therapeutic gain over placebo was generally modest. Furthermore, it remains unclear which organisms are most effective as, for instance, some mainly reduce bloating and flatulence,<sup>209 210 218</sup> whereas others improve bowel frequency,<sup>222</sup> and some have a positive effect on global symptom scores.<sup>214 215 224 226 229 230 233</sup> In some of the better quality trials bifidobacteria, such as *Bifidobacterium infantis* 35624,<sup>215 224 241</sup> *Bifidobacterium lactis* DN 173010<sup>208 225</sup> and *Bifidobacterium bifidum* MIM-Bb75,<sup>226</sup> seem to be advantageous and in others probiotic mixtures appear to be useful.<sup>214 229 233</sup> In only one study was there symptom deterioration<sup>221</sup> although some large, high quality trials have been negative.<sup>221 231 234 235</sup>

#### Diet, fibre, prebiotics and synbiotics

There are few proper randomised, placebo controlled trials of diet modification because of the difficulty in controlling for the placebo effect. One randomised controlled trial showed bran aggravated symptoms;<sup>245</sup> excluding bran should help, and many patients believe this is true.<sup>246</sup> A prebiotic is a product

that, on ingestion, stimulates the growth of beneficial bacteria already present in the host, which promotes the health of the individual.<sup>247 248</sup> A variety of oligosaccharides serve this function and a synbiotic is a combined prebiotic and probiotic. One of the earliest prebiotics was lactulose, an unabsorbable disaccharide laxative that increases the faecal concentrations of *Bifidobacterium* spp.<sup>45 249</sup> as does inulin which, like lactulose, increases flatulence<sup>47</sup> and thus makes it unlikely it will help IBS patients.

To date, there has only been one double blind, placebo controlled trial of a prebiotic in IBS which used a trans-galactooligosaccharide mixture.<sup>250</sup> Compared with placebo this prebiotic reduced symptoms and stimulated the growth of bifidobacteria but clearly more research is required on dosing and the relative merits of other compounds. With regard to synbiotics, there are some studies but their design is not sufficiently robust to draw any firm conclusions<sup>251–255</sup> although the concept of combining a prebiotic and probiotic is theoretically attractive. Thus, attempting to modify the microbiota in patients with functional GI disorders shows some promise. However, we need to know how symptomatic improvement is achieved: is it mirrored by a change in gut microbiota or is some other mechanism involved?

#### CLINICAL GUIDANCE REGARDING MODULATION OF INTESTINAL MICROBIOTA IN IBS

While the science regarding the role of microbiota in FGIDs remains in its infancy, patients are exposed to conflicting claims concerning the symptomatic benefit from modulating gut microbiota. This section aims to help clinicians give the best advice, despite limited evidence (box 4).

Diet profoundly alters the microbiota. Reducing intake of fibre<sup>256</sup> or FODMAPs<sup>257</sup> is one of the simplest and safest ways of altering gut microbiota, which can lead to improvement in bloating and diarrhoea, an effect which may last for years.<sup>258</sup> However, so far the evidence to support widespread use of FODMAP reduction in patients with IBS is limited and comes mainly from one research group. Systematic exclusion diets may also help<sup>258</sup> but are laborious; targeted exclusion of regularly consumed suspects, such as dairy, wheat, fruit and vegetables, may be more practical.

The safety of probiotics in IBS is acceptable but some aggravate symptoms<sup>221</sup> and so patients should be warned of this possibility. At present, the strongest evidence is for *Bifidobacterium infantis* 35624 at a dose of  $1 \times 10^9$  cfu/day taken for at least 4 weeks.<sup>224</sup> It remains unclear who benefits from which variety of probiotic since there are many incompletely answered questions surrounding this therapeutic approach, including:

- ▶ Are single organisms better than mixtures or vice versa?
- ▶ Do some mixtures of organisms contain strains that are competitive or antagonistic without additive effects?
- ▶ Can probiotic foods and drinks be administered simultaneously?

#### Box 4 Diagnostic and therapeutic general recommendations

- ▶ There is currently no clinically useful way of identifying whether the microbiota are disturbed in particular patients with irritable bowel syndrome (IBS).
- ▶ Dietary evaluation and exclusion of possible sources of unabsorbable carbohydrates including fermentable oligo-, di- and mono-saccharides and polyols and excessive fibre could be beneficial in select patients.
- ▶ Probiotics have a reasonable evidence base and should be tried, for a period of at least 1 month, at adequate doses before a judgement is made about the response to treatment.
- ▶ The utility of testing for small intestinal bacterial overgrowth (SIBO) in the setting of IBS remains an area of uncertainty.
- ▶ If SIBO is strongly suspected based on clinical presentation and testing is being considered, using stringent criteria for the glucose breath test or jejunal aspirate appear to be the best tests.
- ▶ Consideration should be given to discontinuing proton pump inhibitors in those with SIBO.
- ▶ There is emerging evidence that non-absorbable antibiotics may have the potential to reduce symptoms in some patients with IBS.

- ▶ What are the best delivery systems—liquids or capsules?
- ▶ How can viability and bioavailability be ensured?
- ▶ What are the optimal dosing regimens and their duration?
- ▶ What is the frequency of host colonisation?
- ▶ Probiotics are not potent pharmacological agents: what patient group should be targeted?
- ▶ Are there any groups of patients where probiotics might be contraindicated such as newborns, immunocompromised or seriously ill individuals?
- ▶ Are there any safety issues about some strains of probiotics, for example, those of *Escherichia coli*?
- ▶ Should different probiotics be given to specific subgroups of IBS patients?
- ▶ Which symptoms of IBS should be the main target for therapy?
- ▶ What are the possible mechanisms behind symptom improvement?
- ▶ How should doctors and patients be advised about their administration?

The considerable acid suppression induced by PPIs may alter upper gut microbiota and can potentially induce IBS symptoms.<sup>92</sup> Thus, it is worth considering PPI discontinuation in selected IBS patients on PPIs for unclear reasons, especially if their symptoms started with PPI therapy.

The most direct way of altering gut microbiota is to use broad-spectrum antibiotics. However, rapid development of antibiotic resistance leads to concerns about using antibiotics in such a ubiquitous and chronic condition. Moreover, it is likely that patients may require repeated courses of therapy, as trial evidence suggests the benefit diminishes by 12 weeks.<sup>189</sup> The best evidence is for rifaximin 550 mg, thrice daily for 2 weeks.<sup>189</sup> The number-needed-to-treat was 11 which should be compared with 4 for 'placebo without deception',<sup>259</sup> 7 for alosetron,<sup>260</sup> 8 for linaclotide<sup>261</sup> and 14 for tegaserod.<sup>262</sup>

Although rifaximin appears to be well tolerated and safe, given its relatively low potency its use should be restricted to difficult cases since its widespread use could promote resistance, such as rifampin-resistant strains of staphylococci.<sup>196</sup> Figure 4 provides a plot chart of currently available strategies for modifying gut microbiota according to the effectiveness and invasiveness of the proposed approach, and general recommendations appear in box 4.

#### CONCLUSIONS AND RECOMMENDATIONS FOR FUTURE RESEARCH AND DEVELOPMENT

Although there is good evidence supporting the concept that the intestinal microbiota is perturbed in patients with FBD, we still lack data on the mechanisms through which host–microbiota interactions underlie pathophysiology and generate symptoms; we need to overcome several boundaries that hold back our knowledge in this field.

The SIBO hypothesis in IBS remains a matter of debate because the breath tests and the small bowel

culture techniques have not been validated. In addition, confounding factors, including the use of antibiotics or PPIs, have not been taken into account in many studies. The wide heterogeneity of FBD and the inter-individual variability of microbiota profiles suggest that larger sample size studies (both in the pathophysiology and therapeutic settings) are of key importance in the future. Attention should be directed to the assessment of correlations between microbiota changes with patient's symptoms. Whenever possible, studies should be stratified by factors known or assumed to affect intestinal microbiota (eg, age, diet, enterotype) and designed to reduce potential confounding factors (eg, antibiotics, probiotics, laxatives, prokinetics, PPI and mesalazine). Although faecal samples are relatively easy to obtain, future work should better characterise microbial populations at the luminal and mucosal level which may differ substantially from faecal microbiota. Host–microbiota interactions are dynamic events and likely influenced by several factors. This suggests that there is a need for longitudinal studies assessing gut microbiota during remission, and symptom flare-ups, stress, infection or following dietary manipulation and the use of probiotics, prebiotics and antibiotics. The effect of bowel transit on microbiota profiles and correlation with symptoms should also be assessed.

One important limitation of available studies is their descriptive rather than mechanistic nature. Accordingly, studies should be directed at clarifying cause–effect relationships between microbiota changes and bowel dysfunction. In this way, microbiota signatures can be developed to help identify IBS biomarkers which might, in turn, offer therapeutic targets. For example, the theory that luminal bacteria may drive low-grade intestinal immune activation should now be substantiated by mechanistic and interventional studies. Data in rodents suggest the existence of a bidirectional interplay between the brain and gut microbiota.<sup>144</sup> In addition, the existence of systemic immune responses to microbial luminal antigens (anti-flagellin antibodies)<sup>148</sup> provides initial evidence that microbial homeostasis may be perturbed beyond the GI tract. These aspects need to be further explored to open new avenues of research in FBD.

Currently, there are promising results suggesting that a subgroup of patients with FBD may respond favourably to a short course of gut-specific antibiotics. However, most probiotic and antibiotic trials are underpowered and suffer suboptimal design. Bloating and flatulence appear to be especially responsive to non-absorbable antibiotics. In order to safely direct these treatment options to the appropriate patients, we need to know more about predictors of treatment responsiveness, the risk of development of antibiotic resistance, the efficacy and safety of re-treatment schedules, and the optimal dosing regimen.<sup>201–202</sup> Further studies should also investigate the mechanism and site of action of non-absorbable antibiotics since amelioration of gas-related symptoms in patients occurred also in patients with no evidence of SIBO.<sup>188</sup> Probiotics seem to have a positive, albeit modest,

effect in both children and adults with FBD, especially IBS. However, head-to-head comparisons between different probiotic products would be useful and future trials need to be large scale, high-quality and use valid end points. Trials should also explore the mechanisms behind symptom improvement.

Faecal transplantation is efficacious in 145/166 (87%) patients with fulminant and refractory *C difficile* infection. This procedure has also been proposed for the treatment of IBS but further research is needed.<sup>263</sup>

In conclusion, a better definition of the role of intestinal microbiota in the pathogenesis and pathophysiology of FBD represents a challenge for the future. Although promising, therapeutic implications will need to be better defined in well-conducted, large clinical trials. A strict cooperation of experienced clinical researchers with microbial ecologists should be considered an important factor for the success of these future studies.

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