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, probiotics, prebiotics, symbiotics and pre-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.1 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, and they are associated with poor health-related quality of life and substantial costs to society.5–9 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.10–12

The human body is inhabited by a complex community of microbes, collectively referred to as the microbiota.13 It is estimated that the human microbiota contains 1014 cells, which outnumber the human cells in our bodies by a factor of ten.14 A vast majority of these are found in the GI tract, with a continuum from 102–109 bacteria per gram of content in the stomach and duodenum to 1011–1012 cells per gram in the colon.15 Moreover, the microbial composition differs between these sites,16 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.17 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.18–19 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,20 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.21

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.22 For instance, many subjects with irritable bowel syndrome (IBS) report symptom onset following an enteric infection.23 There are also studies reporting positive effects of treatments directed at gut microbiota in patients with FBD.24 Moreover, small intestinal
bacterial overgrowth (SIBO)\textsuperscript{25} and altered intestinal microbiota\textsuperscript{26} 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 indepth 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.\textsuperscript{19} 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.\textsuperscript{27} 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.\textsuperscript{28}

Babies are born with sterile intestines but are rapidly colonised by bacteria from their immediate environment, most importantly their mother’s vagina and gut.\textsuperscript{29} 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.\textsuperscript{30} 31 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.\textsuperscript{32} Changes also occur in extreme old age when Bacteroides spp. decrease while Enterococcus spp. and Escherichia coli increase.\textsuperscript{33} 34 Industrialisation has

![Figure 1](image-url)
changed both our diet and microbiota as evidenced by comparing the faecal microbiota of African rural children on 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 (figure 2). Whole grain cereals, resistant starch and low residue diets profoundly alter the microbiota. 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), other studies failed to support these observations. Many dietary prebiotics including oligofructose, lactulose, lupin kernel, inulin-containing juices and arabinoylan-oligosaccharides significantly alter human faecal microbiota. The concept of poorly absorbed but fermentable oligo-, di- and mono-saccharides 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. Accelerating transit with cisapride also increases production of SCFAs, particularly propionic and butyric acids. Acetate, which predominates in the colonic contents, is largely inhibitory. In contrast, propionate and butyrate stimulate motility, activate propulsive ileal motor patterns in humans and ensure that bacteria are propelled from the ileum to the colon. The normal microbiota also strongly influence the mucosal immune system, which is largely inhibitory. In contrast, propionate and butyrate stimulate motility, activate propulsive ileal motor patterns in humans and ensure that bacteria are propelled from the ileum to the colon. The normal microbiota also strongly influence the mucosal immune system. This is largely inhibitory. In contrast, propionate and butyrate stimulate motility, activate propulsive ileal motor patterns in humans and ensure that bacteria are propelled from the ileum to the colon. The normal microbiota also strongly influence the mucosal immune system. This is largely inhibitory.

**Figure 2** Gut microbiota composition in African children living in rural areas with a polysaccharide-rich diet when compared with Italian city children. (Reprinted with permission from Proc Natl Acad Sci USA).
Recent advances in clinical practice

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, metabolomics/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. Draft genomes of cultured gut bacteria can now be produced rapidly and at little cost. 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. 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. These ‘meta-omics’ approaches rely in primary sequencing and annotation data. 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, metabolomics, 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. If SIBO exists, the timing of this fermentation would be altered but the criteria for abnormal tests lack validity (figure 5).

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). 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. Moreover, a recent paper indicated that the small intestinal microbiota are driven by a rapid uptake and conversion of available simple carbohydrates in which Staphylococcus spp. play an important role.

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. 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. 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. 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. 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. 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 (figure 4). Other factors such as antibiotics, probiotics and...
Main features of culture-independent detection methods of gut microbiota

<table>
<thead>
<tr>
<th>Approach</th>
<th>Data generated</th>
<th>Can microbes be identified directly?</th>
<th>Main benefit</th>
<th>Main limitation</th>
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<td>Fast comparison between communities</td>
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<td>Not always</td>
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Recent advances in clinical practice

Infective gastroenteritis is common, with an incidence of 19/100 person years in the UK. 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, while a recent internet survey reported 18%, with around 40% beginning while traveling. The clinical features of post-infectious-IBS are predominantly those of IBS-diarrhoea (IBS-D). 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). Viral gastroenteritis, in keeping with the lesser tissue injury, shows a reduced incidence of post-infectious-IBS compared with bacterial infections in which the strongest risk factors are bacterial toxicity, prolonged duration of diarrhoea, rectal bleeding, and fever. Acute enteritis is associated with a prolonged increase in mucosal cytotoxic T lymphocytes and increase in enterocordine prebiotics, and other dietary items such as FODMAFs 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*. 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. 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 characteristic 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, whose microbiota of IBS patients could be grouped in a cluster which was completely different from that of healthy controls. 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 characteristic of patients. In addition, it should be realised that faecal samples do not necessarily reflect other parts of the GI tract.

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Recent advances in clinical practice

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 H2 gas, resulting from fermentation of the lactulose by intestinal bacteria, and Tc99 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 Tc99 had already reached the meal has reached the caecum. The stylised drawing below shows a representative result from an IBS patient with serial measurements over time. The Tc99 had already reached the caecum in large quantities before the H2 PPM level has reached the threshold for an abnormal test. This demonstrates that the increased H2 production results from fermentation by colonic bacteria, not by abnormal bacteria small intestine (ie, SIBO).

Table 2  Summary of studies culturing small bowel microbiome

<table>
<thead>
<tr>
<th>Study</th>
<th>Number of patients</th>
<th>Sample type</th>
<th>Microbiology results</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drasar and Shiner26</td>
<td>13 Diarrhoea, all investigations negative</td>
<td>Jejunal capsule</td>
<td>No difference from controls; no increased numbers of pathogens or non-pathogens</td>
<td>Possible IBS but not defined as IBS</td>
</tr>
<tr>
<td>Rumessen et al27</td>
<td>60 Patients suspected of SIBO</td>
<td>Proximal jejunal aspirate</td>
<td>15 With no predisposing cause had no evidence of SIBO; of 23 with SIBO, 4 had no predisposing cause</td>
<td>Groups poorly defined, 8 IBS identified and all negative for SIBO; 22 cases considered inconclusive</td>
</tr>
<tr>
<td>Coraza et al28</td>
<td>31 Chronic diarrhoea, no predisposing cause</td>
<td>Proximal jejunal aspirate</td>
<td>10 Had SIBO 1×10^2 cfu/ml or colonic bacteria, 2 IBS, 8 other multiple other diagnoses</td>
<td>IBS not defined, and total IBS not clear</td>
</tr>
<tr>
<td>Bardhan et al29</td>
<td>10 Controls; 4 irritable colon; 22 other</td>
<td>Endoscopic aspirates from proximal jejunum</td>
<td>No positive cultures in irritable colon</td>
<td>Positive cultures in 11 cases, many postsurgical</td>
</tr>
<tr>
<td>Lewis et al30</td>
<td>23 With functional bowel disorders</td>
<td>Duodenal endoscopic aspirate</td>
<td>Mean control count 3.2×10^3 cfu/ml, no anaerobes, no sterile samples</td>
<td>No specific IBS, defined as functional bowel disorders</td>
</tr>
<tr>
<td>Sullivan et al31</td>
<td>7 IBS; 20 controls</td>
<td>Proximal jejunal biopsy using Watson capsule</td>
<td>No differences, flora similar to normal oropharyngeal flora</td>
<td>Colonic pathogen in 2 healthy subjects</td>
</tr>
<tr>
<td>Posserud et al32</td>
<td>162 IBS; 42 controls</td>
<td>Proximal jejunal aspirate</td>
<td>4%×10^3 cfu, same as controls. Subanalysis using 5×10^3, 43% IBS vs 12% controls</td>
<td>No correlation with motor pattern in IBS group</td>
</tr>
<tr>
<td>Kerckhoffs et al33</td>
<td>8 IBS; 9 controls</td>
<td>Proximal jejunal aspirate</td>
<td>No different number diagnosed with SIBO using multiple definitions</td>
<td>No differences also using molecular-based counts</td>
</tr>
<tr>
<td>Choung et al34</td>
<td>148 IBS; 542 ‘other indications to test for SIBO’</td>
<td>Duodenal endoscopic aspirate</td>
<td>2% IBS &gt;10^5 cfu/ml 10% in ‘other’ indications</td>
<td>Retrospective study 18% IBS &gt;0×10^5 cfu/ml</td>
</tr>
<tr>
<td>Pyleris et al35</td>
<td>85 IBS 150 non-IBS</td>
<td>Duodenal endoscopic aspirate</td>
<td>37% IBS &gt;10^5 cfu/ml 15.11% non-IBS</td>
<td>All investigated because of UGI bleed</td>
</tr>
</tbody>
</table>

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.\textsuperscript{138–140} Previous earlier studies in IBS-D suggest impaired SCFA concentrations and production rates in ex vivo incubation, which may also reflect reduced anaerobes.\textsuperscript{141}

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.\textsuperscript{142} A study of children showed that 5 months after \textit{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.\textsuperscript{142}

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.\textsuperscript{143 144} 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 β-defensin-2 peptide) have been detected in patients with IBS and functional diarrhoea and suggest a microbiota-host immune system engagement.\textsuperscript{145 146} 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)\textsuperscript{147} or increased titres of circulating antibodies against components of the indigenous microbiota (ie, antiflagellin antibodies).\textsuperscript{148} Several studies demonstrated low-grade activation of innate and adaptive mucosal immune response in large subgroups of patients with IBS.\textsuperscript{12 149} Increased activated mast cells, CD3+ve, CD4+ve and CD8+ve T cells have been detected in both postinfectious IBS and non-specific IBS.\textsuperscript{12 149}

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.\textsuperscript{150 151} Mast cells were located in closer vicinity to mucosal innervation and correlated with the severity and frequency of abdominal pain in patients with IBS.\textsuperscript{152} 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 sensoryafferent activation and induced visceral hypersensitivity via histamine-1 receptors and protease activated-2 receptors when applied to recipient rats.\textsuperscript{150 151} Intestinal microbiota may well be an active participant in this scenario through stimulation of the immune system,\textsuperscript{153} likely in the subgroup of subjects showing increased epithelial permeability which could 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.\textsuperscript{59–61} 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.\textsuperscript{155} The interplay may be bidirectional as suggested by animal studies showing that the microbiota can affect brain chemistry and behaviour.\textsuperscript{156} 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.

GI DISORDERS MIMICKING AND OVERLAPPING WITH FBD

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

<table>
<thead>
<tr>
<th>Study</th>
<th>Subject</th>
<th>Sample</th>
<th>Method</th>
<th>Patient group</th>
<th>Main finding</th>
<th>Country of study</th>
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<tbody>
<tr>
<td>Balsari et al&lt;sup&gt;96&lt;/sup&gt;</td>
<td>IBS (n=20) Ctrls (n=20)</td>
<td>Faeces</td>
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<td>IBS</td>
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<td>Bilobacteriaceae, Enterobacteriaceae</td>
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<tr>
<td>Si et al&lt;sup&gt;10&lt;/sup&gt;</td>
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<td>Faeces</td>
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<td>IBS</td>
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<td>Malinen et al&lt;sup&gt;99&lt;/sup&gt;</td>
<td>IBS (n=27) Ctrls (n=22)</td>
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<td>IBS</td>
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<td>Mäntö et al&lt;sup&gt;100&lt;/sup&gt;</td>
<td>IBS (n=26) Ctrls (n=25)</td>
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<td>Culture, PCR-DGGE</td>
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<td>Kassinen et al&lt;sup&gt;102&lt;/sup&gt;</td>
<td>IBS (n=24) Ctrls (n=23)</td>
<td>Faeces</td>
<td>GC-profiling + sequencing of 16S rRNA genes</td>
<td>IBS</td>
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<td>R torques 94%, Cl thermosuccinogenes 85%, R bromii-like, R torques 93%</td>
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<td>Rajilic-Stojanovic&lt;sup&gt;103&lt;/sup&gt;</td>
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<td>Krogus-Kurikka et al&lt;sup&gt;105&lt;/sup&gt;</td>
<td>IBS-D (n=10) Ctrls (n=23)</td>
<td>Duodenal mucosa</td>
<td>qPCR</td>
<td>IBS</td>
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<tr>
<td>Lyra et al&lt;sup&gt;106&lt;/sup&gt;</td>
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<td>qPCR</td>
<td>IBS-D</td>
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<td>Tana et al&lt;sup&gt;107&lt;/sup&gt;</td>
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<td>IBS</td>
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<td>IBS</td>
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<td>Carroll et al&lt;sup&gt;109&lt;/sup&gt;</td>
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<td>IBS (n=11) Ctrls (n=22) UC (n=13)</td>
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<td>PCR-DGGE + sequencing of 16S rRNA genes</td>
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<td>Ponnusamy et al&lt;sup&gt;11&lt;/sup&gt;</td>
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<td>Saulnier et al&lt;sup&gt;113&lt;/sup&gt;</td>
<td>IBS (n=22) Ctrls (n=22) (Children)</td>
<td>Faeces</td>
<td>16s Metagenomic sequencing and DNA microarray</td>
<td>IBS</td>
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<td>Rajilic-Stojanovic et al&lt;sup&gt;114&lt;/sup&gt;</td>
<td>IBS (n=62) Ctrls (n=42)</td>
<td>Faeces</td>
<td>Phylogenetic 16S rRNA microarray and qPCR</td>
<td>IBS</td>
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</table>
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. 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’. The underlying mechanisms in humans remain unclear. Mouse models indicate that gluten can induce activation of innate immunity, increased small intestinal permeability, and dysbiosis 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. 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. 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.

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

**TREATMENT IMPLICATIONS: ANTIBIOTICS, PROBIOTICS, PREBIOTICS AND SYNBIOICS**

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, and the fact that antibiotic treatment may increase the development of long-term digestive symptoms after bacterial gastroenteritis, poorly absorbable antibiotics might still have therapeutic potential in this condition. Neomycin was the original choice, although interest is now focused on a non-absorbed derivative of rifampicin called rifaximin.

There are three fully-published, double blind, placebo controlled trials of rifaximin in FBD and the data suggest an improvement in symptoms, especially bloating and flatulence for approximately 10 weeks following treatment.
Recent advances in clinical practice

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 but there remain concerns about antibiotic resistance and possible *Clostridium difficile* infection although so far these issues have not appeared to be a problem.196–200

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.201 202

Probiotics

Probiotics are live microorganisms which when administered in adequate amounts confer a health benefit on the host203 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,59 60 204 205 GI dysmotility,206–210 intestinal permeability,204 211 212 the intestinal microbiota213 214 and immune function,215 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>
<thead>
<tr>
<th>Organism</th>
<th>n</th>
<th>Outcome</th>
<th>Reference</th>
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<tr>
<td>Studies in adult patients</td>
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<tr>
<td><em>S. faecium</em></td>
<td>54</td>
<td>Global score</td>
<td>Gade et al216</td>
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<td><em>Lactobacillus acidophilus</em></td>
<td>18</td>
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<td>Halpern et al217</td>
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<tr>
<td><em>Lactobacillus plantarum 299V</em></td>
<td>60</td>
<td>Flatulence</td>
<td>Nobeak et al218</td>
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<tr>
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<td>20</td>
<td>Pain, ‘all IBS symptoms’</td>
<td>Niedziel et al219</td>
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<td><em>L. plantarum 299V</em></td>
<td>12</td>
<td>Negative</td>
<td>Sen et al220</td>
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<tr>
<td><em>L. plantarum MF1298</em></td>
<td>16</td>
<td>Deterioration of symptoms</td>
<td>Liguere et al221</td>
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<td><em>L. reuteri</em> ATCC 55730</td>
<td>25</td>
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<td>O’Sullivan et al220</td>
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<td><em>L. salivarius</em> UCC4331</td>
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<td>Niv et al223</td>
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<td><em>Bifidobacterium infantis</em> 35624</td>
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<td>O’Mahony et al224</td>
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<td>Digestive discomfort</td>
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<td>Maximum distension &amp; pain</td>
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<td><em>Bacillus coagulans</em> GBI-30, 6086</td>
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<td>Bowel movements</td>
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<td><em>Escherichia coli</em> Nissle 1917</td>
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<td>Treatment satisfaction</td>
<td>Dollet230</td>
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<td>26</td>
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<td>Kraus et al232</td>
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<td>MediLac DS® (x2)*</td>
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<td>Pain</td>
<td>Kim et al234</td>
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<td>Mixture (x4)*</td>
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<td>LAB4 (x4)*</td>
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<td>Pain</td>
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<tr>
<td>VSL#3® (x8)*</td>
<td>59</td>
<td>Global score</td>
<td>Guandalini et al230</td>
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*Number of organisms in a mixture. n, number of randomised subjects.

IBS, irritable bowel syndrome; *L. rhamnosus*, *Lactobacillus rhamnosus*; *L. reuteri*, *Lactobacillus reuteri*; *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.\(^{208–210,212,214–240}\) Unfortunately, their designs vary considerably;\(^{241–244}\) 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.\(^{244}\) 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,\(^{209,210,218}\) whereas others improve bowel frequency,\(^{222}\) and some have a positive effect on global symptom scores.\(^{214,215,220,226,229,230,233}\) In some of the better quality trials bifidobacteria, such as *Bifidobacterium infantis* 35624,\(^{215,224,241}\) *Bifidobacterium lactis* DN 173010\(^{208,225}\) and *Bifidobacterium bifidum* MIM-Bb75,\(^{226}\) seem to be advantageous and in others probiotic mixtures appear to be useful.\(^{214,229,235}\) In only one study was there symptom deterioration\(^{245}\) although some large, high quality trials have been negative.\(^{221,231,234,235}\)

### Diet, fibre, prebiotics and symbiotics

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,\(^{245}\) excluding bran should help, and many patients believe this is true.\(^{246}\) 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.\(^{247,248}\) A variety of oligosaccharides serve this function and a symbiotic is a combined probiotic and prebiotic. One of the earliest probiotics was lactulose, an unabsorbable disaccharide laxative that increases the faecal concentrations of *Bifidobacterium* spp.\(^{45,249}\) as does inulin which, like lactulose, increases flatulence\(^{47}\) and thus makes it unlikely it will help IBS patients.

To date, there has only been one double blind, placebo controlled trial of a probiotic in IBS which used a trans-galactooligosaccharide mixture.\(^{250}\) Compared with placebo this probiotic 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 symbiotics, there are some studies but their design is not sufficiently robust to draw any firm conclusions\(^{251–255}\) 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\(^{256}\) or FODMAPs\(^{257}\) 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.\(^{258}\) 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\(^{258}\) 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\(^{221}\) and so patients should be warned of this possibility. At present, the strongest evidence is for *Bifidobacterium infantis* S5624 at a dose of 1×10\(^8\) cfu/day taken for at least 4 weeks.\(^{224}\) 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.
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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. Thus, it is worth considering PPI discontinuation in selected IBS patients on PPIs for unclear reasons, especially if their symptoms started with PPI therapy.

Probiotics seem to have a positive, albeit modest, culture technique that 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 be the optimal dosing regimen. 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. Probiotics seem to have a positive, albeit modest,

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

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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 endpoints. 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.

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 among experienced clinical researchers with microbial ecologists conducted, large clinical trials. A strict cooperation among experienced clinical researchers with microbial ecologists should be considered an important factor for the success of these future studies.

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