*TOPIC HIGHLIGHT*

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# Intestinal microbiota in pathophysiology and management of irritable bowel syndrome

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## Abstract

Irritable bowel syndrome (IBS) is a functional bowel disorder without any structural or metabolic abnormalities that sufficiently explain the symptoms, which include abdominal pain and discomfort, and bowel habit changes such as diarrhea and constipation. Its pathogenesis is multifactorial: visceral hypersensitivity, dysmotility, psychosocial factors, genetic or environmental factors, dysregulation of the brain-gut axis, and altered intestinal microbiota have all been proposed as possible causes. The human intestinal microbiota are composed of more than 1000 different bacterial species and $10^{14}$ cells, and are essential for the development, function, and homeostasis of the intestine, and for individual health. The putative mechanisms that explain the role of microbiota in the development of IBS include altered composition or metabolic activity of the microbiota, mucosal immune activation and inflammation, increased intestinal permeability and impaired mucosal barrier function, sensory-motor disturbances provoked by the microbiota, and a disturbed gut-microbiota-brain axis. Therefore, modulation of the intestinal microbiota through dietary changes, and use of antibiotics, probiotics, and anti-inflammatory agents has been suggested as strategies for managing IBS symptoms. This review summarizes and discusses the accumulating evidence that intestinal microbiota play a role in the pathophysiology and management of IBS.

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**Key words:** Immunity; Irritable bowel syndrome; Microbiota; Permeability; Probiotics

**Core tip:** Irritable bowel syndrome (IBS) is a functional bowel disorder with multiple pathophysiology, which is not fully understood. Intestinal microbiota has recently been postulated to be involved in the pathophysiology of IBS. Many studies of IBS focus on investigating the efficacy of modulating the microbiota by probiotics and antibiotics. However, the role of the intestinal microbiota in the pathophysiology and management of IBS is not clear. This review provides the accumulating evidence on it.

## INTRODUCTION

Irritable bowel syndrome (IBS) is a functional bowel disorder characterized by abdominal pain or discomfort relieved by defecation, and accompanied by changes in bowel habits such as diarrhea or constipation, which cannot be explained by structural, biochemical, or metabolic abnormalities[1]. The symptoms of IBS have been accounted for as resulting from visceral hypersensitivity, intestinal dysmotility, genetic or environmental factors,
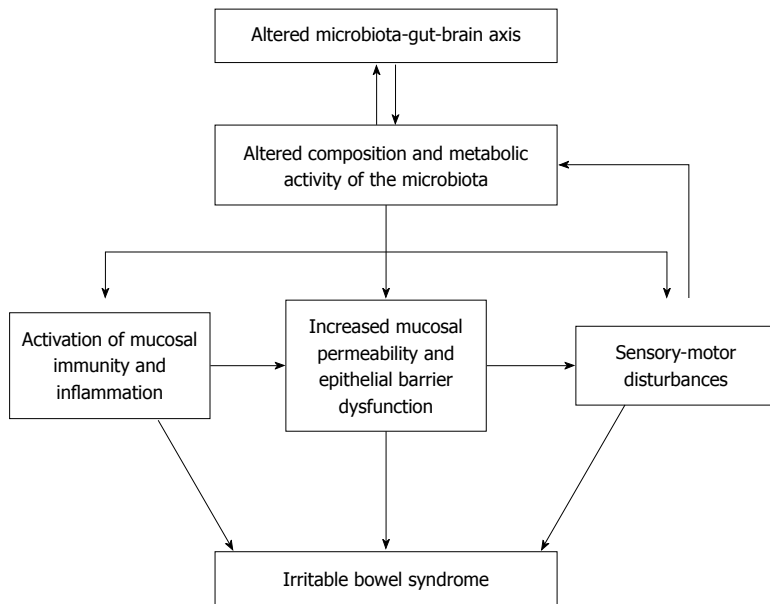
**Figure 1  Putative pathophysiologic role of the microbiota in irritable bowel syndrome.** Intestinal microbiota play a substantial role in irritable bowel syndrome (IBS). Although the microbiota may contribute directly to the symptoms of IBS, it is more likely that altered composition and metabolic activity of the microbiota caused by stress or other psychological disturbances indirectly activate mucosal immunity and inflammation, increase epithelial permeability, and reduce barrier function, thereby activating the sensory-motor dysfunction responsible for a variety of symptoms of patients with IBS.

psychological factors, or a dysregulated brain-gut axis[2]. In addition to these factors, bacterial infection, dysregulated intestinal immune function, and chronic low-grade mucosal inflammation have all been suggested as putative pathogenetic mechanisms, in which the intestinal microbiota might play an important role, but their role in IBS cannot be fully explained (Figure 1)[3,4].

Intestinal microbiota is a collective term for a complex ecosystem of microbes inhabiting the intestine[5]. In the human intestine, this ecosystem may include any one of over 1000 microbial species, and $10^{14}$ cells (*i.e.*, about 10 times more than the number of human cells in the body[6]), containing 150-fold more genes than the human genome[7]. The microbiota can be divided into mucosal and luminal subtypes[8], and it was previously thought to comprise three predominant enterotypes: *Bacteroides, Prevotella,* and *Ruminococcus*[9], although such a strict categorization is no longer widely accepted[10].

To evaluate the composition and metabolic activity of the intestinal microbiota, culture-dependent and -independent tests have been developed[11]. It has been shown that size and diversity of the microbiota increase distally from the upper to the lower gastrointestinal (GI) tract[12] and are modulated by gastric acid, intestinal motility, and the function of the ileocecal valve. Their distribution also varies according to the region of the GI tract with gram-positive facultative anaerobic bacteria in the proximal small intestine and gram-negative anaerobes in the distal small intestine. Although the composition and diversity of the microbiota are genetically controlled from birth and become stable after weaning and throughout life, qualitative and quantitative changes can occur over the longitudinal and cross-sectional axes of the intestine: changes in bacterial enzymes and metabolic activity, as

well as in microbial populations. The composition and metabolic activity of the microbiota vary between, but also within, individuals due to many factors including mode of delivery at birth, diet, sanitation, antibiotics, and ageing[13]. At birth, contamination from the vaginal canal provides the intestine with the maternal microbiome, while during a delivery by cesarean-section, the gut comes into contact with commensals from the skin and the surgical environment[14]. The composition of the microbiota can also be altered by the feeding method: bifidobacteria increase in breast-fed babies (*i.e.*, babies receiving a high-carbohydrate and high-fiber diet), and *Bacteroides* spp. increase in formula-fed babies (babies receiving a high-fat diet)[15]. Lastly, it can vary across geographical regions, *e.g.*, between rural Africa and urban Europe[16].

The intestinal microbiota is essential for maintaining individual health, including normal GI function. In this context, its main functions are metabolic, protective, and trophic: it can help to digest and absorb nutrients, and produces a variety of beneficial compounds such as short-chain fatty acids (SCFA)[17], it can act as a barrier against pathogens by adhering to the mucosa, generating immune responses, and interacting with components of the epithelial layer, it can also influence the differentiation and proliferation of the intestinal epithelial cells and the development of the enteric immune system.

In parallel with the beneficial effects of microbial activity on the gut, bacterial fermentation may give rise to large amounts of gas and thus contribute to the symptoms of bloating, flatulence, and abdominal distension, which are commonly reported by patients with IBS[18]. An association between the microbiota and IBS has been supported by the evidence of modulation of mucosal immunity: IBS symptoms were found to be more frequent

after an episode of gastroenteritis, and some IBS symptoms were found to improve after antibiotic treatment targeting the intestinal microbiota[19]. This putative link was also demonstrated in studies of probiotics, which modulated the intestinal microbiota in IBS patients. Finally, mucosal immunity-gut microbiota-brain axis is being suggested as a possible pathway for the development of IBS due to altered intestinal microbiota. This review article explores the role of the microbiota in the pathophysiology and management of IBS, and provides a comprehensive summary of the evidence for the concept of IBS as a microbiota-related disorder.

Despite the large volume of studies of the intestinal microbiota, our understanding of its role in health and disease is still in its infancy. In studying the microbiota, culture-based methods are being replaced by advanced, culture-independent, molecular techniques. However, these two approaches are complementary: culture studies of fecal matter or colonic mucosa are valuable for identifying functional groups and for selective enumeration, whereas advanced molecular study are a powerful tool for monitoring changes in microbial composition. The molecular methodology includes sequencing of the small-subunit ribosomal RNA genes through amplification of nucleic acids extracted from fecal or mucosal samples, fingerprinting methods such as denaturing gradient gel electrophoresis, targeted methods such as fluorescence in situ hybridization and quantitative PCR, new high-throughput sequencing, and 16S rRNA-based microarraying[20].

## PUTATIVE PATHOPHYSIOLOGIC ROLE OF INTESTINAL MICROBIOTA IN IBS

### Alteration of the microbiota-gut-brain axis

The microbiota in the gut can be altered by brain function, and microbial alteration can, in turn, influence brain function. It is evidenced by the finding that patients with IBS frequently have accompanying psychological disorders, such as anxiety or depression, and those with psychological stress are more likely to develop post-infectious (PI)-IBS. This connection between the microbiota, the gut, and the brain in IBS postulates the existence of a bidirectional, homeostatic network, and it is an exciting area of ongoing research.

Animal studies have demonstrated the influence of the intestinal microbiota on brain development. Brain dysfunction in Germ-free (GF) mice was reported, including an exaggerated hypothalamic-pituitary response to mild stress[21], more exploratory and risk-taking behavior[22], and altered brain chemistry and memory, indicative of impaired hippocampal development[23]. Brain chemistry and behavior were also influenced by altered microbiota; a study showed that transient alteration of the microbial composition by diet provoked exploratory behavior, accompanied by changes of in the levels of brain-derived neurotrophic factor in the specific regions of the brain such as hippocampus and amygdala[24]. The gut microbiota and the brain may be communicated by

neural, metabolic (bacterial and host), immunologic, or endocrine pathways[25]. The neural pathways was first suggested in animal models; anxiety-related behavior was reduced after probiotic treatment, provided vagus nerve integrity was maintained[26,27]. Metabolic pathways were revealed in a study that brain function and behavioral changes were closely associated with bacterial metabolites such as SCFAs (which comprise most of the circulating organic acids) and tryptophan metabolites[28,29]. A role of immunologic pathways was demonstrated in animal and human studies showing that certain psychological disorders were associated with pro-inflammatory cytokines, whose levels had been altered by manipulating the composition of the microbiota[30-32]. Endocrine pathways in microbiota-gut-brain axis were suggested in a study showing that the endocrine structure and function of the GI tract which secrets a variety of hormones such as cholecystokinin and serotonin [5-hydroxytryptamine (5-HT)] were reduced in GF rats[33].

Likewise, the intestinal microbiota can be affected by signals from the central nervous system produced in response to stress or psychological disturbances. Stress can change GI motility and secretions, which alter the microbial habitat. The microbial habitat may also be altered by changes in gene expression of some microbial species. Conversely, the intestinal microbiota can influence neurotransmitters like norepinephrine, dopamine, and serotonin in the brain, and activation of the hypothalamic-pituitary-adrenal axis is also thought to be involved in the microbiota-gut-brain axis.

As a result of alteration of the microbiota in this axis, mucosal immunity may be activate and thereby epithelial barrier function can be disrupted, which could contribute to the visceral hypersensitivity and dysmotility in IBS. Furthermore, the intestinal microbiota may not only release metabolites but also induce the formation of host-derived immune mediators, thereby affecting the enteric nervous system both directly and indirectly. However, much about the role of the microbiota-gut-brain axis in IBS remains poorly understood.

### Dysbiosis: quantitative and qualitative changes in the microbiota

**Altered composition of the intestinal microbiota**: Intestinal microbiota can be grouped into luminal and mucosal microbiota. It is generally accepted that the composition of the luminal and mucosal microbiota differs between patients with IBS and healthy controls, and the composition may also vary according to the subtype of IBS[34], although studies of the intestinal microbiota have been as diverse and complex as the microbiota itself, with inconsistent and conflicting results[29,35-44] (Table 1). According to both the early culture-based and the more recent advanced molecular studies, it was found in IBS that the proportions of specific bacterial groups were altered, the diversity of microbial populations was reduced, and the degree of variability in the microbiota composition was different. The findings included decreased levels of

**Table 1  Summary of studies of the intestinal microbiota in patients with irritable bowel syndrome**

| Ref. | Subject (*n*) | Method | Finding |
|---|---|---|---|
| Si *et al*[35] | IBS (25) | Culture | Decreased *Bifidobacterium* |
| | Control (25) | | Increased *Enterobacteriaceae* |
| Malinen *et al*[36] | IBS (27) | qPCR | Decreased *Lactobacillus* in IBS-D |
| | Control (22) | | Increased *Veillonella* in IBS-C |
| Mättö *et al*[37] | IBS (26) | Culture | Increased coliform and aerob to anaerob ratio |
| | Control (25) | PCR-DGGE | Temporal instability |
| Codling *et al*[38] | IBS (41) | PCR-DGGE | No difference in fecal/mucosal |
| | Control (33) | | Temporal instability |
| Ponnusamy *et al*[39] | IBS (11) | DGGE | Increased diversity in *Bacteroidetes*, *Lactobacillus* |
| | Control (8) | qPCR-16sRNA | |
| Tana *et al*[29] | IBS (26) | Culture | Increased *Lactobacillus* and *Veillonella* |
| | Control (26) | q-PCR | |
| Lyra *et al*[40] | IBS (20) | qPCR | Increased *Ruminococcus* torques and decreased |
| | Control (15) | | *Clostridium thermosuccinogenes* in IBS-D |
| Krogius-Kurikka *et al*[41] | IBS (10) | 16S rRNA | Increased *Proteobacteria* and *Firmicutes* |
| | Control (23) | sequencing | Decreased *Actinobacteria* and *Bacteroidetes* |
| Kerckhoffs *et al*[42] | IBS (41) | FISH | Decreased *Bifidobacterium* |
| | Control (26) | qPCR | |
| Kassinen *et al*[43] | IBS (24) | 16S rRNA | Decreased *Collinsella aerofaciens*, *Clostridium cocleatum*, and *Coprococcus* |
| | Control (23) | sequencing | *eutactus* |
| Maukonen *et al*[44] | IBS (24) | PCR-DGGE | Decreased *Clostridium coccoides* |
| | Control (16) | | Temporal instability |
| Jeffery *et al*[46] | IBS (37) | 16S rRNA | Increased ratio of *Firmicutes* to *Bacteroidetes* |
| | Control (20) | pyrosequencing | Clustering in IBS |

DGGE: Denaturing gradient gel electrophoresis; FISH: Fluorescent in situ hybridization; IBS: Irritable bowel syndrome; qPCR: Quantitative polymerase chain reaction.

fecal lactobacilli and bifidobacteria, increased levels of facultative anaerobic bacteria dominated by streptococci and *Escherichia coli* (*E. coli*), increased ratios of *Firmicutes*: *Bacteroidetes* and higher counts of anaerobic organisms (such as clostridium)[44,45]. In addition, the microbiota of IBS patients reportedly belonged to entirely different enterotypes than those of healthy controls[34,46]. These inconsistent and sometimes conflicting results are thought to be due to the use of a single fecal sample irrespective of the fluctuating symptoms of IBS.

**Altered metabolic activity of the intestinal microbiota:**
Intestinal microbiota may produce excessive amounts of gas by fermenting poorly absorbable carbohydrates (*e.g.*, the so-called FODMAPs, fermentable oligosaccharides, disaccharides, monosaccharides and polyols), which may cause abdominal pain, bloating, flatulence, and distension in IBS. Additionally, altered fermentation of poorly absorbable carbohydrates could increase the production of SCFAs, which would then lead to release of 5-HT from the intestinal mucosa[47]. In fact, increased numbers of acetic and propionic acid-producing bacteria (*Veionella* and *Lactobacillus* spp) were reported in patients with IBS[29]. It has been demonstrated that the release of 5-HT initiated high-amplitude, propagated colonic contractions, accelerated intestinal transit, and increased gut motility[47,48], all of which may contribute to IBS symptoms, suggesting that fermentation products play a potential role of in contributing IBS symptoms.

However, considering the large variability due to different methodologies of microbiota studies, and indi-

vidual differences in relation to dietary, genetic and geographical factors, as well as heterogeneity of the disease, these results should be cautiously interpreted. Research on the luminal and mucosal microbiota is still in infancy, and further studies using advanced techniques such as 16s rRNA and DNA sequencing are needed to improve our understanding of the microbiota changes in IBS.

***Activation of mucosal immunity and inflammation in IBS***
The altered composition and metabolic activity of the intestinal microbiota found in IBS may be associated with activation of mucosal immunity and inflammation. Changes in the intestinal microbiota were observed after an episode of infective gastroenteritis with subsequent antibiotic use. In fact, some patients start to report IBS symptoms following such episodes[49], which suggests an association between IBS and activation of mucosal immunity and inflammation caused by altered microbiota. Chronic low-grade mucosal inflammation has been frequently observed in many studies of IBS patients and in animal models of IBS[50-56].

The intestinal microbiota plays an essential role in the development, functioning, and regulation of both intestinal and systemic immunities. By interacting with the microbiota, the intestinal (or enteric) immune system, composed of innate and adaptive immunity, helps to maintain normal GI function[57]. In IBS patients, however, the interactions between enteric immunity and commensal and/or pathogenic microbes were found to be dys-regulated. Under normal conditions, intestinal microbes are recognized via their ligands, identified by toll-

like receptors (TLRs) on intestinal immune cells. Expression of TLRs in the colonic mucosa of IBS patients was found to be increased[58], as was the level of circulating antibodies such as antiflagellin antibodies[59]. Together, these findings suggest that in IBS, bacterial components such as lipopolysaccharides (LPS) and flagellin are recognized more frequently due to the increased TLRs and circulating antibodies. In addition, one of the antibacterial proteins, β-defensin-2, was found to be elevated in IBS[60]. These increased interactions of immunologic components with the microbiota could eventually lead to the mucosal inflammation in IBS.

Mucosal inflammation provoked by dysregulated innate and adaptive enteric immunities has been observed in many studies of IBS[61,62]. The numbers of activated mast cells were shown to be increased in the colon of IBS patients, and also to be in close proximity to enteric nerves, which correlated well with IBS symptoms[63], although this increase was specific to diarrhea predominant IBS (IBS-D)[52], and varied according to the region of the intestine[64]. In addition to mast cells, lymphocytes (CD4+ and CD8+ T cells) were also found to be elevated, suggesting that they may play a role in IBS, although there are some inconsistencies[50,55,56,65]. Immune alterations associated with IBS were also found in IgA-producing B cells[66], IgG+ B cells[67], and in the levels of pro- and anti-inflammatory cytokines such as tumour necrosis factor-alpha (TNF-α) and interleukin (IL)-10, IL-6, and interferon-γ in the intestinal mucosa of IBS patients[68]. Similarly, in the peripheral blood, levels of pro-inflammatory cytokines such as IL-6, IL-1β, and TNF-α were higher in patients with IBS than in controls, but the levels varied according to IBS subtype[69]. It is thought that mucosal inflammation and activated immunity in IBS may lead to increased permeability of the intestinal mucosa, and may thus induce abnormal sensory and motor function, which could contribute to the symptoms of IBS. However, the association between activated immunity and the intestinal microbiota is not clearly established, and further studies in this area are warranted.

### Altered mucosal permeability and the epithelial barrier in IBS

The activation of mucosal immunity and inflammation driven by the altered microbiota in IBS may increase mucosal permeability and impair epithelial barrier function. The intestinal epithelium functions not only as an exchanger, absorbing fluid and nutrients, but also as a protective barrier against pathogens. It is covered with a thick layer of mucus, composed of a complex mixture of glycoproteins, mucins, bactericidal enzymes, and secretory immunoglobulin A (IgA). Alterations to the epithelial barrier observed in IBS have included increased mucosal permeability, increased expression of specific proteins, *e.g.* MUC20 (gene involved in the production of mucin) and PARM1, and increased fecal excretion of the antibacterial protein β-defensin-2[60]. Increased mucosal permeability in the small intestine was observed

in patients with IBS-D[70], and it was associated with the expression and distribution of tight junction proteins; lower levels of the protein zonula occludens (ZO)-1 were found in IBS patients than controls[71,72]. Elsewhere, increased permeability along with mast cell infiltration into the colon was found to be associated with the severity of IBS symptoms[73]. Both the increased permeability and symptoms of IBS were improved by lactic acid bacteria, suggesting that there may be an association between an altered epithelial barrier and IBS symptoms[74]. It is worth noting that one study of gut permeability found that the increase was limited to the colon[75], whereas another IBS study reported that the expression and distribution of ZO-1 was altered in the jejunum[76]. Generally, this increase in gut permeability was found to be associated with bacteria-related protease activity and its receptors in the intestinal epithelium[77]. It is also thought that a single-nucleotide polymorphism in the gene encoding the tight junction protein, E-cadherin, may increase the risk of developing PI-IBS[78]. On the other hand, some bacterial metabolites produced by the intestinal microbiota were found to improve epithelial barrier function[79]. It has also been suggested that the barrier dysfunction with increased mucosal permeability in IBS may also be associated with visceral hypersensitivity[80].

### Sensory-motor disturbances caused by intestinal microbiota

In addition to the mucosal inflammation of the gut that may affect sensory-motor and secretory functions, neuronal structure, and neurotransmitter release in the gut[81], the intestinal microbiota can directly affect intestinal sensory-motor functions[82]. Alterations in the microbiota induced by antibiotic treatment were found to precipitate visceral hypersensitivity, which was restored by probiotic treatment[83]. Probiotic treatment was also found to reduce sensation of pain via the enteric nerve in a model of visceral pain induced by colorectal distension[84]. A similar level of pain modulation was also achieved by inducing the expression of opioid and cannabinoid receptors[85]. With respect to motor disturbances, it has been reported that colonic motor function was enhanced by supernatants from the *E. coli* strain Nissle 1917, and that this was mediated by stimulation of smooth muscle cells[86]. Also, probiotic treatment was found to increase small-intestinal motor function in rats[87]. Furthermore, transplantation of healthy human fecal microbiota into GF mice increased their colonic motility and shortened GI transit, which was closely associated with the type and amount of carbohydrates in the diet[88]. The beneficial effects of the microbiota on motility were shown to be region-specific with migrating motor complex velocity increased in the jejunum but decreased in the colon[89]. These interactions between intestinal microbiota and GI sensory-motor function may be related to IBS, although the exact mechanism of the interactions is not well understood.

It seems that normal GI motility relies on TLR4 sig-

naling stimulated by the microbiota. It was demonstrated that mice lacking TLR4, which is frequently stimulated by bacterial LPS, exhibited longer GI transit times and reduced abundance of colonic nitrergic neurons[90]. In addition to the microbiota itself, the metabolites from bacterial fermentation may also exert an effect on GI motility. One of the colonic metabolites, $CH_4$, was shown to delay intestinal transit[91], $H_2S$ was shown to inhibit the contraction of intestinal smooth muscle[92], SCFA, to stimulate colonic transit by triggering 5-HT release[47], and tryptamine from tryptophan, to increase intestinal contractions[93]. Other bacterial metabolites that may be related to GI motility include bile acid metabolites[94] and ligands of GABA receptors with a suppressive effect on GI motility[95]. While the microbiota may affect gut sensory-motor function, the reverse may also be true: the microbial ecosystem in the gut may be disturbed by accelerated or decelerated GI transit[88]. It is thought that the changes in GI transit may alter the flow rate of intestinal contents and thereby affect the environment for resident bacteria, which then impinges on both the organizational structure and the gene expressed in the microbiota.

# MODULATION OF THE INTESTINAL MICROBIOTA FOR MANAGING IBS SYMPTOMS

## Dietary modifications
An association between diet and symptom development in IBS is reported frequently but its mechanisms are not clearly defined. Some of the proposed causative factors include hypersensitivity and/or allergic reaction to specific foods, and alterations of the habitat and metabolic activity of the intestinal microbiota. Diet is thought to be a powerful factor influencing the composition and metabolic activity of the microbiota in an individual. The composition of the microbiota in babies change after weaning, and in adults it varies according to geographic regions due to differences in the food consumed, the type of meat consumed, and cooking methods (whether the food is fried, baked or boiled). Therefore, any dietary strategy aimed at modifying the microbiota should be matched to the individual because different microbial species are responsive to different kinds of dietary components.

However, whether a change in the diet can directly affect the microbiota in IBS is not clear. This is partly due to the lack of well-designed, controlled trials that investigate the effects of diet on IBS. Although specific diets, *e.g.*, the FODMAPs diet, have been shown to provoke IBS symptoms in some patients, not all studies regarding the effects of exclusion diets on the symptoms of IBS are completely reliable due to a variety of confounding factors, including a high placebo effect. Nevertheless, it can be speculated that in some IBS patients, intake of certain foods may provoke abnormal fermentation due to aspects of the composition of their intestinal microbiota

and that the composition of the microbiota in those patients could be changed to normal by excluding the symptom-provoking foods.

Dietary fiber stimulates the production of SCFAs by mixing with microbes and enzymes. In a healthy gut, these by-products can improve the function and homeostasis of the GI tract. Although it has been suggested that some patients with IBS may benefit from dietary fiber, many patients report an increase in abdominal distension and bloating as a result of fermentation of the fiber. It may be that water holding properties of fiber and its ability to accelerate intestinal transit may alter the habitat for the microbiota and therefore indirectly affect its composition and metabolic activity.

It seems that individualized advice on dietary consumption of non-digestible carbohydrates in the management of IBS, as the inter-individual differences in the response of the microbiota lead to different responses to changes in diet[96].

## Antibiotics
Antibiotic treatment in IBS assumes that small intestinal bacterial overgrowth (SIBO) plays an important role in the development of IBS. Despite the limited validity and lack of standardization of the methods used to evaluate SIBO, treatment with non-absorbable antibiotics such as rifaximin has yielded a therapeutic benefit. Double-blind, placebo-controlled trials of rifaximin in IBS yielded an improvement in IBS symptoms, which correlated well with the reduced excretion of hydrogen in the breath[97,98]. These findings together with the positive effects of other antibiotic treatments, suggest that a short course of poorly absorbable antibiotics may be of some use in the management of IBS symptoms in some patients. However, data on the long-term effects of antibiotics in IBS are limited. Furthermore, information on the optimal dose of antibiotics, and predictors of treatment success and failure are needed to confirm the benefit of this type of treatment[99].

## Probiotics
**Effects of probiotics**: By adhering to intestinal epithelial cells and competing for nutrients and space, probiotics can protect against pathogens. This protective effect of probiotics has been demonstrated *in vitro* using intestinal cell lines with lactobacilli, bifidobacteria and *E. coli* subspecies[100-102]. In addition, probiotics can improve mucosal barrier function and thereby prevent pathogens from increasing intestinal permeability[103,104]. Intestinal permeability can also be increased by stress, which may facilitate the subsequent translocation of pathogenic bacteria. However, it was observed that the increase in intestinal permeability caused by stress was inhibited by lactobacilli[105-107]. In addition, lactobacilli increased levels of bacterial fermentation products such as SCFAs (acetic, propionic and butyric acids) and thereby acidifying the colon, which subsequently increased the numbers of *Bifidobacterium* and *Lactobacillus* species and decreased

clostridia[108]. In addition to these roles, probiotics were also shown to modulate immunity in animals with experimentally-induced colitis[109,110]. Furthermore, they were shown to reduce visceral hypersensitivity by increasing the expression of opioid and cannabinoid receptors in the intestinal mucosa[85].

However, regarding the effect of probiotics on IBS symptoms, the mechanism is not clearly defined. It is possible that probiotics may not only modulate gut dysmotility and hypersensitivity but also have anti-inflammatory properties. It was found that probiotic treatment attenuated intestinal dysmotility in a mouse model, induced intestinal cell mediators related to reduced hypersensitivity such as cannabinoid and opioid receptors, and normalized the ratio of cytokines IL-10/IL-12 in the systemic circulation.

**Probiotic studies in IBS**: A majority of studies of probiotics in IBS have been performed to evaluate their effect on either overall or specific IBS symptoms. Although most of them have used *Lactobacillus* or *Bifidobacterium* species, single strains or combinations of multiple strains have also been used with multiple doses (from $10^6$/mL to $10^{10}$/mL) and for variable durations. Similarly, primary and secondary outcomes in those studies were evaluated using variable factors such as abdominal pain, symptom severity, quality of life, and global IBS symptoms. On balance, these studies found a therapeutic benefit, *i.e.*, improvement in symptoms of bloating, flatulence, bowel frequency, and in global symptoms, although there are some inconsistencies between specific studies. In particular, beneficial effects of probiotics were reported in a well-designed study using bifidobacteria such as *Bifidobacterium infantis* 35624[30,111], *B. lactis*, *B. animalis* DN173010, and *B. bifidum* MIMBb75[112]. Symptom improvement was also reported in studies using probiotic mixtures such as *Escherichia coli* (DSM 17252) and *Enterococcus faecalis* (DSM 16440)[113], and *Lactobacillus rhamnosus* GG, *L. rhamnosus* LC705, *Bifidobacterium breve* Bb99 and *Propionibacterium freudenreichii* ssp. s*hermanii* JS[114,115]. By contrast, negative results were reported in studies using other probiotic combinations[116], such as *Lactobacillus paracasei* spp. *paracasei* F19, *L. acidophilus* La5 and *Bifidobacterium lactis* Bb12[117,118], and *Lactobacillus plantarum* MF1298[119].

In recent studies, it was found that 4-wk treatment with probiotics improved IBS symptoms and altered composition of the microbiota as well[120], and that probiotic treatment in IBS patients reduced the genus Bacteroides to the levels of healthy controls and also improved global IBS symptoms[121]. However, as indicated in several meta-analyses, the previous studies of probiotics in IBS fail to report whether symptom improvement was accompanied by a change in the microbiota or not. Furthermore, many systematic reviews pointed out several study limitations including heterogeneity, inadequate statistical methods, and possible publication bias. Examples of heterogeneity include differences in types, doses, and delivery of probiotics[122-125], which may have produced different outcomes. Therefore, despite the reported benefits of probiotics in IBS, there are many aspects of potential treatment regimens that are yet to be established, such as adequate dosage, treatment duration, choice of species for each individual or symptom of IBS, target symptoms for probiotics, and probiotic formulation. Future studies should aim to identify which species, strains, and doses of probiotics provide the optimal therapeutic benefit to individual patients with IBS, and which specific symptoms of IBS should be the target of probiotic treatment.

## CONCLUSION

Intestinal microbiota can play a substantial role in IBS. Although the microbiota may contribute directly to the symptoms of IBS, it is more likely that altered composition and metabolic activity of the microbiota caused by stress or other psychological disturbances indirectly activate mucosal immunity and inflammation, increase epithelial permeability, and reduce barrier function, thereby activating the sensory-motor dysfunction responsible for a variety of IBS symptoms. Therefore, our knowledge of the link between the microbiota and IBS may enable us to treat focusing on the possible mechanism of this disorder; Dysbiosis may be restored by probiotic or antibiotic treatment and also by diet modification. Activation of mucosal immunity and inflammation can be treated by immune-modulating agents. Increased intestinal permeability and barrier dysfunction can be a potential therapeutic target of probiotics. However, the microbial pathophysiology of IBS is not clearly understood, as microbiota alterations in IBS might be either a cause of IBS or a consequence of intestinal secretion and motility changed by IBS. Furthermore, due to the heterogeneity of IBS studies as well as IBS itself, there has been variability in the results of studies. Therefore, objective diagnostic modalities in IBS are warranted, and further studies using advanced molecular techniques are needed.

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