# MICROBIOTA

SPECIAL OVERVIEW ISSUE | JULY 2022

BIOCODEX
Microbiota Institute

# SUMMARY

# EDITO

**Special "overview" issue**

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> "THIS ISSUE GIVES YOU A CLEAR IDEA OF THE VARIETY AND DIVERSITY OF RESEARCH PROJECTS CURRENTLY UNDERWAY."

Dear colleagues,
As you may have noticed from the various issues of Microbiota Magazine, the gut microbiota has long since ceased to be an obscure, non-mainstream, area of research monopolized by a handful of passionate and determined researchers. In 2011, 622 scientific publications relating to the gut microbiota were cited on PubMed. By 2021, this figure had risen to 11,743.

A clear sign of dynamic medical research, this proliferation of publications is also our source of material. Our ambition in each issue of Microbiota Magazine is to give you the most accurate picture possible of progress in research on the gut microbiota.

Thanks to its exhaustiveness and the contribution of leading specialists, the "overview" article has become the magazine's focal point. Recent readership surveys have confirmed it as one of the most popular sections of the magazine.

Given your interest, we decided it would be helpful to bring together the last six overview articles published. This "overview of overviews" gives you a clear idea of the variety and diversity of research projects currently underway. Whether it is the involvement of the gut-brain axis in irritable bowel syndrome, the role of the gut microbiota in *Clostridioides difficile* infections, the metabolism of drugs by the gut microbiota, the dialog between the gut microbiota and host immune responses to infection, or more recently the links between the gut microbiota and autism, Microbiota Magazine gives you a representative and up-to-date summary of the latest findings on the gut microbiota's role in health. Enjoy.

Harry Sokol, Emmanuel Mas

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# GUT MICROBIOTA AND *CLOSTRIDIUM DIFFICILE*

***C. difficile*** **infection (CDI) has become in recent years a clinical and socioeconomical burden worldwide, due to its increase in morbidity, severity, mortality, and likelihood to recur. There is a considerable involvement of gut microbiota in CDI, for many reasons. First, most risk factors associated with the development of CDI, including the overuse of broad-spectrum antibiotics or proton pump inhibitors, are associated with an imbalance of gut microbiota. Moreover, specific microbiota modulators are involved in the prevention (specific probiotics) or treatment (fecal microbiota transplantation) of CDI. In this paper, we will review epidemiology, risk factors, and approved therapies of CDI, with a microbiota-centric view.**

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*Clostridioides difficile* (*C. difficile*, previously called *Clostridium difficile*) is a gram-positive, spore-forming, obligate anaerobe. Spores allow *C. difficile* to persist in environments, and to be spread from infected subjects. Under specific circumstances (*e.g.,* antibiotic-driven dysbiosis), spores are driven to germination in the large bowel, and present in a vegetative form that leads to clinical infection (*Clostridium difficile* infection [CDI]). In the infection phase, *C. difficile* produces two toxins, enterotoxin A and cytotoxin B that both cause damage to colonocytes and trigger the inflammatory response, leading to a variety of clinical pictures, from mild colitis to pseudomembranous colitis and toxic megacolon [1].

In recent years, CDI has become a considerable healthcare and economical burden in most countries. Studies from the United States report an incidence of nearly 453,000 cases and of nearly 29,000 CDI-related deaths in 2011, while the incidence in Europe is 124,000 cases/year, with nearly 3,700 deaths/year. Increased morbidity, hospitalization length and mortality, contribute to the considerable economic burden of CDI, which accounted for nearly $ 5 billions in the US in 2011, and for nearly € 3.7 billions in Europe in 2013 [2, 3]. These figures show that the CDI incidence has risen worldwide, for several reasons. First, the increased use of antibiotics, which are a known as risk factors for CDI development. Furthermore, the spreading of specific ribotypes (mainly the virulent ribotype 027, but also the 017 in Asia, the 018 in Italy, the 17,621 in Eastern European countries, 24,422 in Oceania) has let CDI clusters develop. Additionally, there was also an increased number of diagnoses, due to the development of highly sensitive diagnostic tests (*e.g.*, PCR), and the risen awareness of CDI among healthcare professionals.

Overall, the main cause of the overall increase in CDI incidence appears to be the increased rate of recurrences. From 2001 to 2012, the annual incidence of recurrent CDI has increased by nearly 189%, while the increase in overall CDI incidence in the same time period was nearly 43% [2]. As recurrent infection is less likely than first episode to be cured by antibiotics, it is associated with longer hospitalization, increased morbidity and mortality too.

Despite this increase in diagnoses, the misdiagnosis/underdiagnosis of CDI is still relevant, as observed in the EUCLID study.

This finding suggests that a considerable number of patients with CDI is still not diagnosed, increasing the risk of disease diffusion.

CDI is widely known to be the main cause of healthcare-associated infectious diarrhoea, but recent evidence suggests that its diffusion in the community settings is growing. To date, nearly 25%-35% of CDI cases are acquired in community, probably due to several fecal-oral transmission pathways (*e.g.*, zoonosis and food).

Nosocomial CDI, a community-acquired CDI, appear to differ for several characteristics. First, nosocomial patients are more likely to present with a severe clinical picture, while community patients can even be asymptomatic carriers, increasing the risk of CDI spreading. Moreover, community-based CDI is known to spread also among patients without standard risk factors.

## RISK FACTORS FOR *C. DIFFICILE* INFECTION

Although the exact pathogenic pathways of CDI are not yet clarified, several risk factors have been identified over time [4]. Their knowledge is relevant as the management of modifiable risk factors is a prevention measure against CDI. Most relevant risk factors include older age, use of antibiotics, proton pump inhibitors, and others (**Figure 1**).

### ANTIBIOTICS

If antibiotics remain today essential molecules in the therapeutic arsenal, it is also necessary to take into account their undesirable effects on the gut microbiota, as a considerable body of evidence supports the association between their use and many dysbiosis-associated diseases, including CDI [5].

The use of systemic antibiotics is the most relevant modifiable risk factor for the development of CDI. Healthy gut microbiota can determine the successful colonisation of the large bowel by C. difficile or not, by direct and indirect pathways. In principle, the imbalance of healthy gut microbiota by broad-spectrum antibiotics may bring several consequences that drive to CDI.

First, antibiotics may kill commensal bacteria that may have a direct action against *C. difficile* (by secreting a number of bacteriocins) and also compete with the pathogen for nutrients (*e.g.*, sialic acid and succinate). Moreover, there is also an indirect protective role of commensal bacteria through the regulation of bile acids.

Recently, *Clostridium scindens* was associated with resistance to *C. difficile* colonisation. It has a bile acid inducible operon which is able to encode dehydroxylating enzymes that convert primary bile acids into secondary bile acids. Primary bile acids promote the germination of *C. difficile* spores, while secondary bile acids are able to inhibit this process [6].

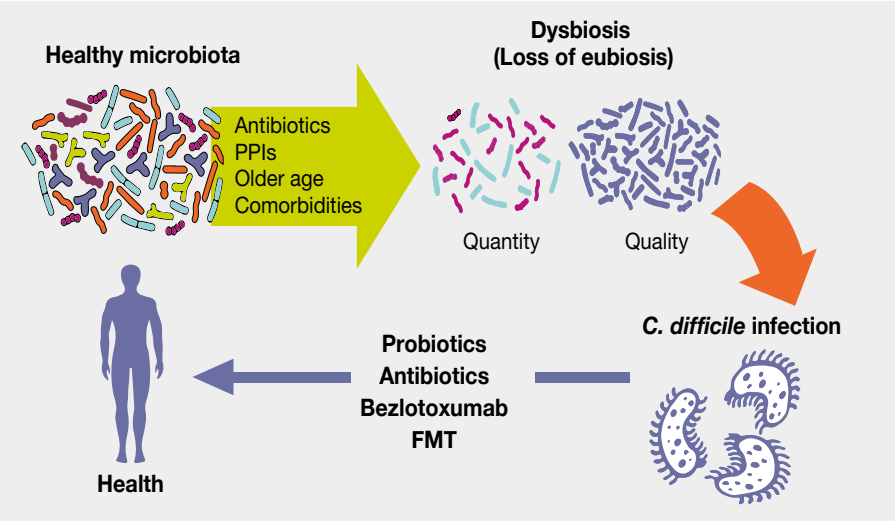
As a corollary of this evidence, patients with recurrent CDI are known to have an imbalanced microbial profile, with higher relative abundance of detrimental bacterial families as *Enterobacteriaceae* and *Veillonellaceae* and lower relative abundance of beneficial families, including *Ruminococcaceae, Bacteroidaceae* and *Lachnospiraceae*.

A number of systematic reviews, alone or with meta-analysis, have assessed the relevance of different antibiotic classes in CDI development. In the earliest meta-analysis (1998), antibiotics use was associated with a 6-fold increase in the risk of developing CDI, and the highest risk was observed for fluoroquinolones, clindamycin, cephalosporins. Moreover, the use of antibiotics was found to be an independent predictor of CDI recurrence (relative risk 1.76). One of the key factors to prevent CDI is represented by the antibiotic stewardship approach, so the knowledge of the CDI risk for different antibiotic classes is of paramount importance (**Table 1**).

The use of the following antibiotics is associated with a 2-fold higher risk of CDI among inpatients: clindamycin, cephalosporins, carbapenems, fluoroquinolones, trimethoprim, sulphonamides. In the community setting, respectively, antibiotics were found to have different risk levels for CDI development or recurrence, in-

▼FIGURE 1

**A microbiota-centric view of *C. difficile* infection**



▼TABLE 1
**Antibiotics predisposing to CDI**

| Frequently | Infrequently | Rarely |
|---|---|---|
| Ampicillin and amoxicillin | Tetracyclines | Parenteral aminoglycosides |
| Cephalosporins | Sulfonamides | Bacitracin |
| Clindamycin | Erythromycin | Metronidazole |
| | Chloramphenicol | Vancomycin |
| | Trimethoprim | |
| | Quinolones | |

cluding: clindamycin (risk increased of 8 to 20 times), cephalosporins and fluoroquinolones (3-5 times increase), macrolides (2-3 times increase) [5].

## GASTRIC ACID SUPPRESSION

Proton pump inhibitors (PPIs) are largely used worldwide for several upper gastrointestinal disorders, including gastroesophageal reflux disease, hiatal ernia, gastritis, *H. pylori* infection (together with antibiotic eradication therapy), peptic ulcer disease.

Overall PPIs are considered safe drugs. However, a large body of evidence shows that the use of PPIs is significantly associated with the development of CDI.

In principle, PPIs can increase the risk of *C. difficile* colonization by several pathways, including reduced acid production that can lead to small intestinal bacterial overgrowth and dysbiosis, and increase of bile salts that can promote the germination of *C. difficile* spores. Finally, there is no clear evidence if increased gastric pH is a safer environment for spores [6].

The clinical evidence of a significant association between PPIs and CDI comes from several systematic reviews and meta-analyses, with odds ratios ranging from 1.26 to 2.34, based on different reports (from 3 to 67, according to different meta-analyses).

Most evidence is heterogeneous and comes from observational cohorts, so potentially confounding factors, including other drugs and co-morbidities, could reduce the quality of this finding. However, the association between PPIs and CDI kept significant even after stratification for antibiotic use, both in cohort studies and in case-control reports.

The detrimental role of PPI was found to be stronger toward community-associated CDI, suggesting that there is a chronic overuse in communities rather then in hospitals.

Specifically, PPIs have been associated not only with CDI overall, but also with recurrent CDI by several meta-analyses (including from 3 to 16 studies), with odd ratios ranging from 1.52 to 2.51, although definitions of recurrence varied significantly among studies.

## ADVANCED AGE

Advanced age is one of the best known risk factors for primary CDI and recurrent CDI.

Established evidence shows that CDI rates are much higher in adults over 65 years than in younger population. In a meta-analysis of 33 studies, age older than 65 years was identified as an independent predictor of recurrent CDI (relative risk 1.63).

However, age is a considerable confounder, as the use of several drugs that promote CDI, such as antibiotics or PPIs, is more common in older age. Increasing evidence suggests that the microbiota of elderly patients is less healthy (in terms of reduced microbial diversity and increase of opportunistic species) than normal, supporting again the role for microbiota imbalance in CDI [7].

## OTHER DISORDERS

The association between CDI and selected comorbidities has also been explored systematically. In a systematic review, significantly higher risk of CDI was found for inflammatory bowel disease (OR 3.72), kidney insufficiency (OR 2.64), hematologic malignancies (OR 1.75), and diabetes mellitus (OR 1.15). This was especially true for community-acquired CDI [7].

# THERAPEUTIC MANAGEMENT OF CDI

## CONVENTIONAL TREATMENT OF CDI

Traditionally, metronidazole and vancomycin have been the most common treatment options for CDI, being used both as first line options, while only vancomycin was recommended, as tapered or pulsed regimen, to treat recurrent disease [8].

However, in recent years CDI has become more cumbersome to treat. In particular,

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metronidazole was shown to achieve lower cure rates than vancomycin, so that vancomycin has been preferred to metronidazole also in primary infection. Overall, also vancomycin is losing its efficacy, and the rates of recurrent disease have grown. Morover, hypervirulent strains of *C. difficile* have emerged, specifically the ribotype 027, which is less responsive to standard antibiotic therapy and is associated with more severe clinical pictures [8].

In recent years fidaxomicin, a narrow spectrum antibiotic, was shown to be superior than vancomycin in treating CDI recurrences. However, its high costs and the recent evidence of its inferiority compared with fecal microbiota transplantation (FMT) in treating recurrent CDI are potential limitations to its widespread use [9].

## THERAPEUTIC MICROBIOTA MODULATORS: PROBIOTICS AND FECAL MICROBIOTA TRANSPLANTATION

Generally, probiotics are considered a reliable option to restore healthy gut microbiota after a dysbiotic event, *e.g.*, antibiotic treatments. Overall, some probiotics are known to be effective against antibiotic-associated diarrhea (AAD), which is a common adverse event of antibiotic regimens [10-12]. In a metanalysis of 21 randomized trials, *Saccharomyces boulardii* decreased significantly the risk of AAD (risk ratio: 0.47) [11].

As CDI is basically a subgroup of AAD, the efficacy of probiotics in preventing CDI was then investigated. Recently, a Cochrane review has shown, in a meta-analysis of 23 trials, that probiotics are both safe and effective for preventing CDI [13]. However, only specific probiotics, including *Saccharomyces boulardii, Lactobacillus casei, a mixture of L. acidophilus* and *Bifidobacterium bifidum*, and a mixture of *L. acidophilus, L. casei* and *L. rhamnosus*, have been found to be effective in preventing primary CDI after antibiotic therapies. In particular, S. boulardii was effective in preventing CDI in a cohort of elderly hospitalized patients, with likely saving of money. Indeed, a Canadian study showed that the use of preventative probiotics was able to save $ 518/patient than usual care, and to reduce the risk of CDI [11]. However, further, larger studies are needed to confirm the role of specific probiotics in CDI prevention.

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FMT is the infusion of stools from healthy donors in the gut of a recipient to cure a dysbiosis-related disorders. To date, several systematic review and meta-analyses have shown that FMT is highly effetive in curing recurrent CDI (up to 90% cure rates).

Based on this outstanding evidence, scientific societies have included FMT among the treatment options for recurrent CDI [14, 15]. FMT is also known to increase overall survival and decrease hospitalization length in patients with recurrent CDI [16]. Although FMT has been increasingly standardized over years, is still underdiffused worldwide. Future microbiota-based approaches that will guarantee a widespread diffusion of FMT include capsulized FMT and microbiota-based drugs.

## CONCLUSION

**CDI is a burdensome disease that occurs mainly in patients with several risk factors, most of which are associated with gut microbiota imbalance, including antibiotic overuse, proton pump inhibitors, and older age. Also from a microbiological point of view, the microbial profile of patients with CDI is characterized by a deep imbalance of gut microbiota. Therapeutic microbiota modulators have been shown to be effective in preventing (specific probiotics, some *Lactobacillus* strains and *S. boulardii*) or curing (FMT) recurrent CDI, paving the way for a microbiota-based approach for the management of this disorder.**


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# THE GUT MICROBIOTA AND DRUG METABOLISM

**The gut microbiota transforms the chemical structures of ingested compounds, including orally-administered small molecule drugs. This metabolism, which can vary substantially between patients, impacts drug efficacy in both positive and negative ways, and can also influence toxicity. Over the last 10 years, there has been a growing appreciation of the potential contribution of gut microbiota drug metabolism to inter-individual variability in patient drug response. Here, we review this topic, with a focus on recent advances and their potential future impact on patient care and drug discovery.**

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The trillions of microorganisms that inhabit the human gut possess a greatly expanded set of genes compared to the host genome. Many of these genes encode protein-based catalysts, or enzymes, that enable gut microbes to perform a wide range of chemical reactions, expanding the chemistry associated with the human body. A hallmark of gut microbial metabolism is its variability; just as the composition of the microbiota differs between individuals, so too can the metabolic capabilities of this community. As we continue to identify associations between the gut microbiota and health and disease outcomes, it is becoming increasingly important to characterize microbial metabolic transformations at a molecular level.

One prominent activity associated with the gut microbiota is the ability to chemically modify the structures of small molecule drugs [1]. Orally administered drugs encounter gut microbes either prior to absorption in the small intestine or in the large intestine if they are poorly orally bioavailable. Orally administered or injected drugs, or drug metabolites, also reach the microbiota if they undergo biliary excretion into the intestine. Because a drug's pharmacological activity directly arises from its chemical structure, microbial metabolism can have a large effect on drug action.

## EFFECTS OF GUT MICROBIAL DRUG METABOLISM

Gut microbial metabolism has various downstream consequences for drug action and efficacy (**Figure 1**). As the early examples of azo drugs illustrate, microbial metabolism of 'prodrugs' (inactive precursors) may be required to generate the active pharmacological agent. This knowledge has inspired the rational design of additional strategies for targeted drug release in the large intestine that rely on microbial metabolic activities.

Studies of gut microbial drug metabolism began over 80 years ago with the discovery that the early antibiotic Prontosil, an azo compound that is inactive toward bacterial isolates but displays efficacy in vivo, underwent reduction by the gut microbiota to give the active agent sulfanilamide. Additional examples of gut microbial drug metabolism were uncovered throughout the intervening years, often prompted by observations of varying efficacy or toxicity in patients. Importantly, despite this history, such activities still are not typically considered in drug development or administration.

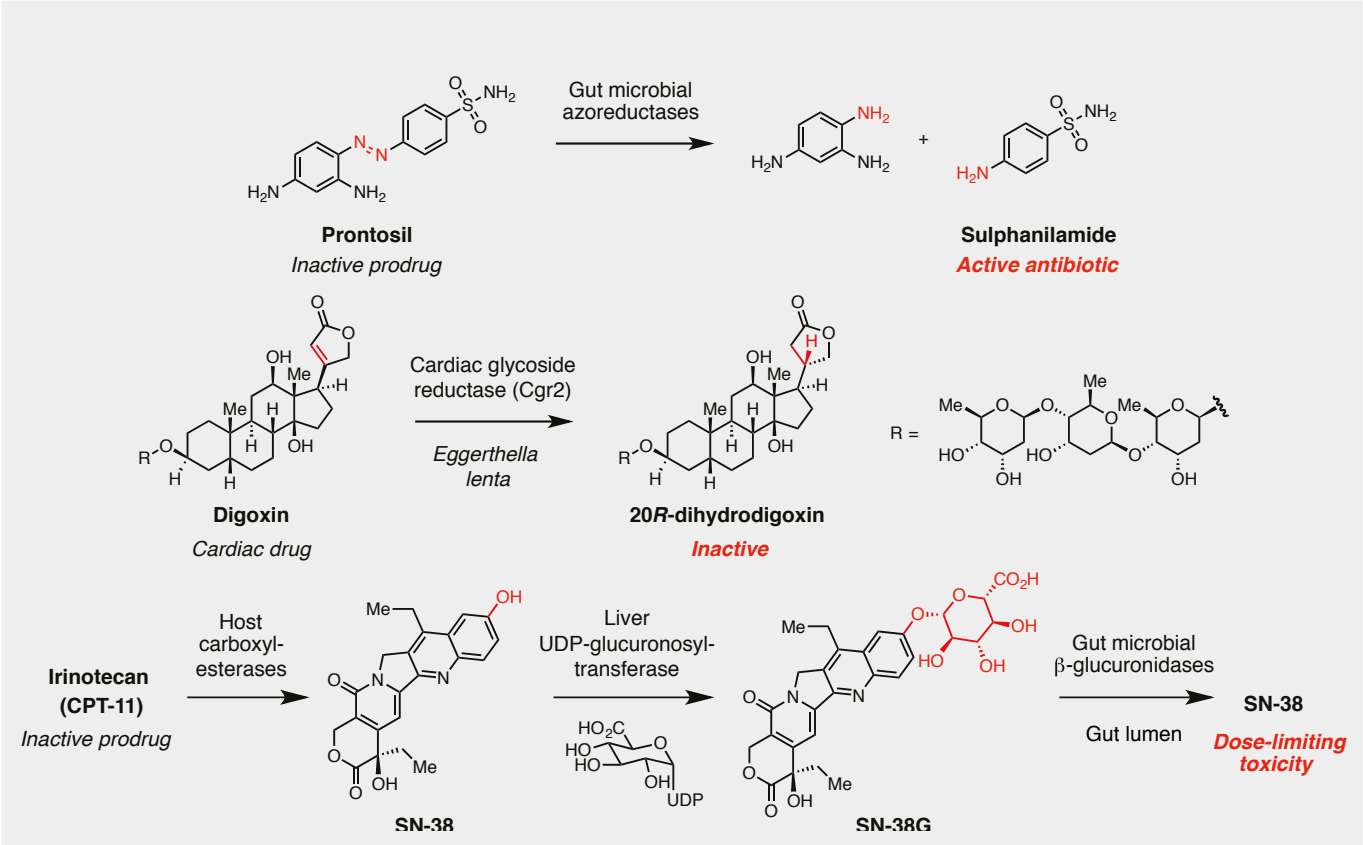
Metabolism by the gut microbiota can also have negative effects on drug activity by disrupting interactions with intended host targets. One example is the natural product-based cardiac medication digoxin. In 5-10% of patients, the gut microbiota reduces the α, β-unsaturated lactone ring of digoxin to give dihydrodigoxin. This subtle modification, which is performed by the gut bacterium *Eggerthella lenta*, greatly reduces the binding affinity for digoxin's target Na+/K+ ATPase, resulting in a loss of efficacy [2]. Another prominent example is the front-line Parkinson's disease treatment L-dopa. Metabolism of L-dopa to dopamine by host enzymes in the brain is critical for alleviation of symptoms. Gut microbial metabolism of L-dopa also produces dopamine [3,4]. Because dopamine generated in the periphery cannot cross the blood brain barrier, this activity may reduce the amount of L-dopa that reaches the brain.

Finally, in addition to reducing activity, the chemical modifications installed by gut microbes can produce unwanted toxicity. For example, gut microbial metabolism was implicated in the lethality of co-administering the antiviral medication sorivudine with fluoropyrimidine chemotherapeutics. This outcome was traced to gut microbial metabolism of sorivudine to bromovinyluracil. This metabolite inhibits a key host enzyme involved in detoxifying 5-fluorouracil, increasing its concentration to lethal levels.

The chemistry of gut microbial drug metabolism, which tends to be reductive and hydrolytic, is often unique from that of host transformations, which involve oxidation of drugs and conjugation with more polar metabolites to facilitate excretion. Microbial metabolism often has opposing effects on drug availability, prolonging circulation in the body. However, microbial drug transformations do not have to be distinct to impact drug action; recent studies of the anti-viral drug brivudine suggest such activities can affect drug pharmacokinetics even when they are identical to host metabolism [5].

▼FIGURE 1

**Gut microbial drug metabolism has varying effects**



An important characteristic of gut microbial drug metabolism is its variability across patients. This phenomenon has its origins in the variability of the gut microbiota. Though some metabolic activities are found in many organisms, others are carried out by a small, low abundant subset of the gut community. Metabolism can vary between individual strains of the same species, as even closely related bacteria can have large differences in their genomes. It is therefore perhaps unsurprising that community composition is often a poor predictor of metabolism, and metabolism of individual drugs can be extensive in some individuals and absent in others. This variation likely has important but incompletely understood consequences for patients taking a range of small molecule drugs.

## UNDERSTANDING DRUG METABOLISM AT A MOLECULAR LEVEL

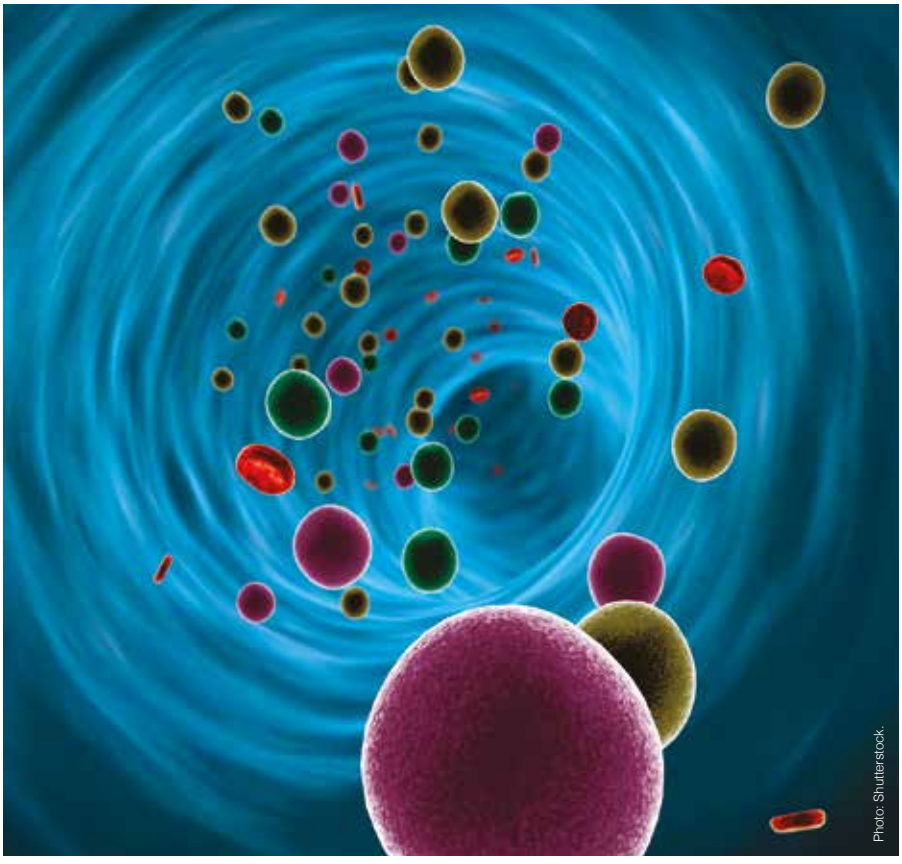
In order to fully understand gut microbial drug metabolism, it is necessary to link individual activities with microbes, genes, and enzymes. Identifying specific drug-metabolizing microbes is typically needed to enable downstream mechanistic studies. This may be accomplished through screening available gut microbial isolates or isolating metabolizing organisms directly from complex gut microbiota samples. An important next step is connecting transformations of interest to genes and enzymes. This is crucial for studying metabolism in complex gut communities, as the genes encoding metabolic enzymes allow detection and prediction of individual activities in microbial genomes and microbiome sequencing data. Linking drug metabolism to microbial genes can be accomplished in multiple ways, including rationally searching genomes for enzymes with the requisite catalytic capabilities, using RNA-Seq to identify genes that are specifically upregulated in response to a drug, and using comparative genomics to associate genes with metabolic capabilities.



An estimated 70% of gut microbial diversity is uncultivated, making it challenging to characterize their activities. Donia and co-workers used functional metagenomics, which introduces DNA isolated directly from a complex microbiota into a heterologous host, to identify a hydrocortisone-metabolizing gut bacterial enzyme [6]. Cholesterol metabolizing enzymes were also recently discovered in uncultured gut bacteria by correlating the presence of microbial genes in microbiomes with metabolomics data [7]. Both strategies may be useful for investigating drug metabolism by uncultured organisms.

## IDENTIFYING NEW METABOLIC ACTIVITIES

Until 2019, approximately 60 examples of gut microbial drug metabolism were reported. Two recent studies leveraged approaches from high-throughput screening and experimentation to perform large scale surveys of gut microbial drug metabolism, greatly expanding the scope of known transformations. Goodman and co-workers screened 76 human gut bacterial isolates for their ability to metabolize 271 small molecule drugs and found that two thirds of the drugs were depleted by at least one organism [8]. The Donia group performed an analogous screen of 575 drugs using a patient gut microbiome sample ex vivo and uncovered 45 new transformations [6]. These efforts suggest the scope of drugs subject to metabolism may be larger than previously known; however, the vast majority of these newly reported activities have not yet been confirmed in vivo, so their relevance for patients is unknown.



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## MANIPULATING GUT MICROBIAL DRUG METABOLISM

Once the gut microbiota has been found to transform a small molecule drug, a logical next step is to ask how this activity may be controlled, both to assess the consequences of metabolism for drug action and to improve patient therapy should metabolism prove detrimental. Various methods have been employed to achieve this goal. Using gnotobiotic animal models (germ-free animals colonized in a controlled manner with a defined microbiota), one can compare communities containing either drug metabolizing gut strains or deletion mutants missing specific activities. The utility of this approach was nicely illustrated by the Goodman lab's studies of brivudine [5].

However, genetic manipulation is challenging in native, complex microbial communities, prompting evaluation of alternative approaches. One potential strategy is to leverage knowledge of gut bacterial physiology to guide manipulation of the gut environment via dietary interventions. For example, digoxin Turnbaugh and co-workers noted that the presence of L-arginine downregulates drug metabolism by *E. lenta* [2]. They then showed that administering protein-rich diets to gnotobiotic mice colonized with *E. lenta* reduced drug inactivation *in vivo*.

Another exciting strategy is to identify small molecules that inhibit the activity of gut microbial drug metabolizing enzymes, as pioneered by the Redinbo lab in their studies of irinotecan metabolism. Irinotecan is a prodrug that is metabolized by host cells to the active topoisomerase inhibitor SN-38. SN-38 is metabolized by the host via glucuronidation, which produces an inactive conjugate (SN-38G). This metabolite is excreted into the intestine, where the glucuronide is removed by gut bacterial β-glucuronidase (GUS) enzymes. This reactivation causes dose-limiting gastrointestinal tract toxicity. The Redinbo group used high-throughput screening to identify selective inhibitors of gut bacterial GUS enzymes, and found they prevented the severe side effects caused by irinotecan in a mouse model [9]. Subsequent work revealed that these compounds increase the efficacy of irinotecan by limiting its toxicity [10]. Together, this work has provided exciting proof-of-concept for therapeutically targeting gut bacterial metabolism and has prompted additional inhibitor discovery efforts.

## FUTURE FRONTIERS

The successful development of GUS inhibitors as therapeutic candidates highlights one way in which gaining a molecular understanding of gut microbial drug metabolism could benefit patients. Another area that could be transformed by this knowledge is precision medicine. With an understanding of how specific therapeutics are metabolized by gut microbes, physicians could one day use microbiome sequencing data or microbiota-based diagnostic assays in deciding whether and how to prescribe particular medications.

Our growing appreciation of gut microbial drug metabolism may also influence the drug discovery process itself. Due to past associations with toxicity and side effects, many functional groups known to be transformed by gut bacteria are typically avoided by medicinal chemists. One could imagine uncovering new, unanticipated transformations early in drug development by screening individual gut microbes or complex patient communities for metabolism ex vivo, similarly to how drug candidates are typically tested for metabolism by host enzymes. Differences in gut microbiota composition and functions between animal models and humans should be taken into account in preclinical and clinical studies. Finally, it may be advisable to incorporate microbiome sample collection and analysis for drug metabolism into clinical trials. Correlating metabolism with differences in toxicity or efficacy might help in interpreting the results of such trials and defining target patient populations.

### CONCLUSION

**In summary, the last decade has witnessed great leaps in our understanding of the molecular mechanisms underlying gut microbial drug metabolism and its consequences for drug efficacy. Further efforts to explore this exciting research area are poised to advance precision medicine and drug discovery.**

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# CROSSTALK BETWEEN THE GUT MICROBIOTA AND THE HOST'S IMMUNE RESPONSE TO COMBAT INFECTIONS

**The fact that living beings have evolved over millions of years in complex environments occupied by microbial ecosystems has shaped symbiotic relationships regulated by the immune system. The new sequencing techniques have revolutionised our knowledge and have shown that each individual hosts a microbiota which is unique to him, as is its role in the physiology of the host and in numerous diseases such as infections. The interaction between the gut microbiota and the immune system starts during foetal life. Their mutual and constant exchanges shape both the immunity of the host and also the gut microbiota resulting in protection from infection and numerous diseases. Indeed, the specific organisation of the microbiota - separated from the host by a single layer of cells - constitutes a particular challenge for the immune system, the role of which is to recognise "non-self" as a potential sign of infection and thus trigger the immune system cascades. For this reason, the continuous exchanges with the microbiota have a significant impact on the immune system of the host. The immune response, which must be tolerant towards the microbiota, also has an impact on the composition and function of this microbiota.**

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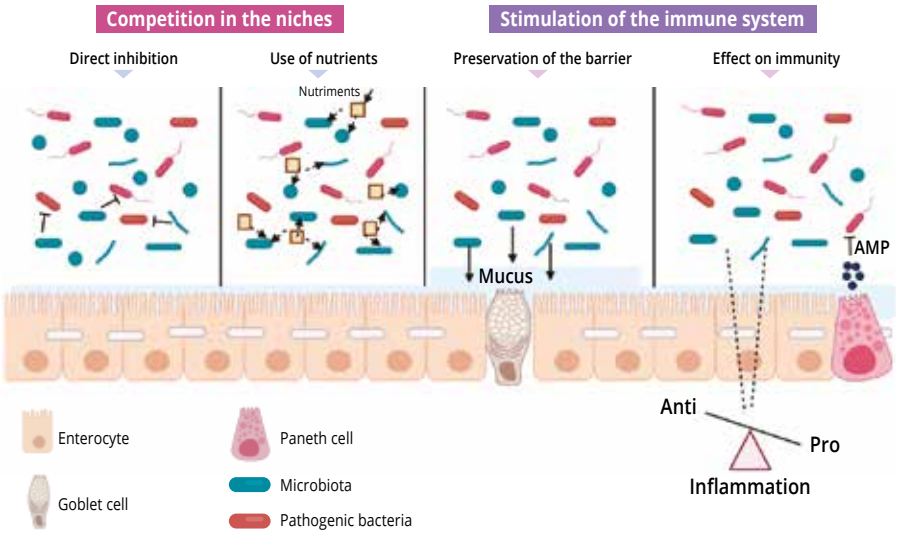
## GUT MICROBIOTA AND THE INTESTINAL BARRIER

The gut microbiota is an initial barrier protecting the intestinal mucosa from pathogens. This complex ecosystem inhabits the gastrointestinal tract where it remains stable and limits access to the intestinal niches and to the nutrients required for the multiplication of exogenous bacteria by the phenomenon called "colonisation resistance" [1] (**Figure 1**). The enterocytes, which provide a physical barrier between the intestinal lumen and the host, absorb water and nutrients and secrete antimicrobial peptides, AMPs (RegIIIγ, β-defensins and cathelicidin) [2]. By the recognition of microbe-associated molecular patterns, (MAMPs) by specific receptors (including the Toll-Like-Receptors, TLR), these cells will be able to transduce the signal to cytokines and chemokines thus signalling infection and recruiting immune cells (**Figure 2**). Paneth cells also participate in colonisation resistance by secreting AMPs (lysosyme, α-defensins, RegIIIγ) [2].

The goblet cells – mucus-secreting – and the M cells have gatekeeping action, transporting antigens, intact and captured at random in the intestinal lumen arising from

▼FIGURE 1

**Functions of the gut microbiota which contribute to colonisation resistance**

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commensal bacteria or pathogens or dietary antigens. These will then be prepared by the dendritic cells (DC) and presented to the adaptive immune system. This function is vital to intestinal tolerance and the induction of mucosal immune responses [2]: there therefore is a constant balance between pro- and anti- inflammatory responses (**Figure 2**). In particular, this was demonstrated in mice models of induced colitis and in TLR receptor-deficient mice: the absence of microbiota or recognition of this reduces the proliferation of intestinal epithelial cells or barrier repair [2]. Lastly, the mucus also provides protection by capturing AMPs, which act to prevent the pathogens from reaching the epithelium. In the model of Muc2-deficient mice (Muc2 is the gene coding for one of the proteins making up the mucus), an increase in the translocation of commensal bacteria is observed and these animals develop intestinal inflammatory diseases [3].

## CROSSTALK BETWEEN THE GUT MICROBIOTA AND THE INNATE IMMUNE SYSTEM

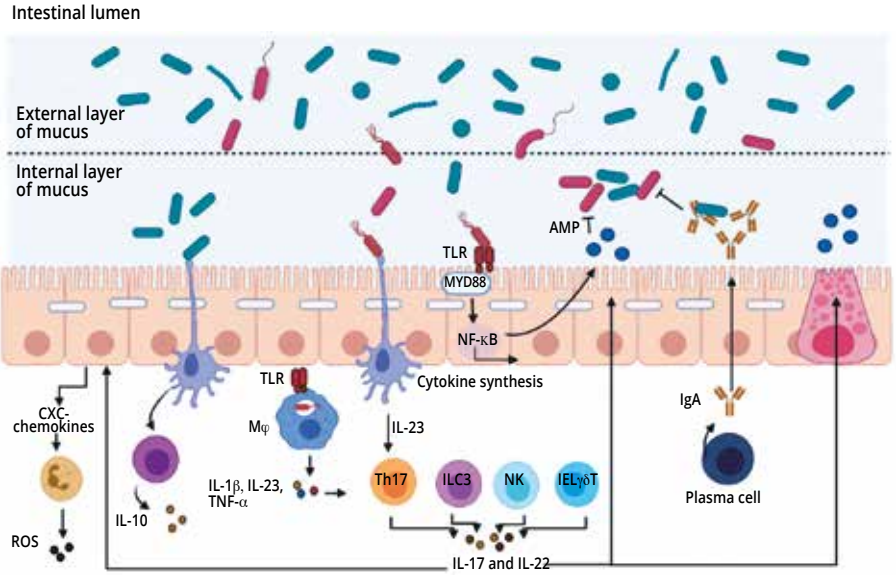
Among the players of the innate immune system which participate in intestinal homeostasis, antigen-presenting cells (APC), such as the macrophages (Mφ) and the DCs have a major role. The Mφ and the DCs synthesise IL-10 and thus promote differentiation of Treg [4] and the maturation of the Th17 lymphocytes via the implication of IL-22, a cytokine known to stimulate the synthesis of AMPs [6]. To come back to the DCs, these, by extending their dendrites between the epithelial cells, are able to phagocyte the bacteria present in the intestinal lumen. These commensal bacteria are then transported to the mesenteric lymph nodes to induce the production of IgA secreted by the plasma cells [1].

The innate lymphoid cells (ILC) also play an important role in intestinal homoeostasis; this is related to their capacity to initiate and direct intestinal immune responses. More specifically, the type 3 ILCs (ILC3) have a unique place in the interaction with the gut microbiota. By synthesising IL-22, these cells stimulate the production of mucus, AMPs and the secretion of chemokines and recruitment of polymorphonuclear (PMN) cells (**Figure 2**) [1].

of commensal bacteria: the segmented filamentous bacteria (SFB). These have the particular ability to adhere to the intestinal epithelial cells causing active stimulation of the immune system [5] (**Figure 3**). A study shows that colonisation of mice by these SFB, induces the differentiation of Th17 thus resulting in protection from *Citrobacter rodentium* (the murine equivalent of EPEC and EHEC). It has been suggested that this protection is due to the capacity of the SFB to cause Th17 to stimulate the synthesis


▼FIGURE 2

**Response of the immune system to infections**

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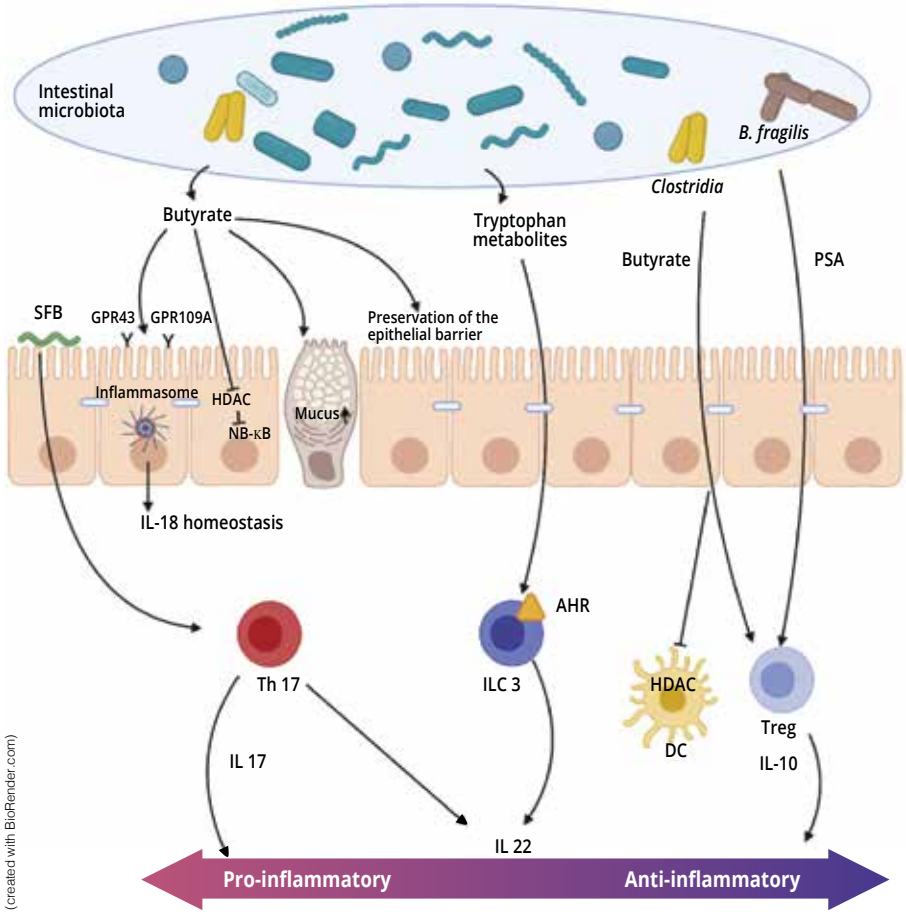
▼FIGURE 3

**Metabolites produced or synthesised by the gut microbiota and their impacts on immune responses**




## CROSSTALK BETWEEN THE MICROBIOTA AND THE ADAPTIVE IMMUNE SYSTEM

The final maturation of the adaptive immune system is characterised by the colonisation of the intestinal mucosa by mature effector T-lymphocytes with inflammatory properties (Th17), T-lymphocytes with anti-inflammatory properties (Treg) and B-lymphocytes (**Figure 2**). Besides effects on the macrophages and the differentiation of the Th17 cells, the SFB also stimulate the development of the lymphoid follicles and participate in the differentiation of the B-lymphocytes to IgA-producing plasma cells the action of which is the containment of pathogenic bacteria in the mucus [5]. Other commensal bacteria can stimulate adaptive immune responses: a mixture of 17 *Clostridia* strains isolated from a human faecal sample and introduced in mice induced an anti-inflammatory response by stimulating the Treg [7]. *Faecalibacterium prausnitzii* has also been identified for its anti-inflammatory action *in vitro* and *in vivo* by acting on the NF-κB factor, DCs and Mφ which secrete IL-10 and enhance differentiation of Treg to the detriment of Th17 [8]. Of the Bacteroidetes, *Bacteroides fragilis* and *B. thetaiotaomicron* have also been described as exerting anti-inflammatory activity. *B fragilis* synthetises a polysaccharide A (PSA) that prevents pro-inflammatory IL-17 production and stimulates the anti-inflammatory of secretion IL-10 (**Figure 3**). In a specific model of *Helicobacter hepaticus*-induced colitis, PSA stimulated the development of lymphoid follicles, stimulated Treg lymphocyte cells and protected the mice [9].

## MICROBIAL METABOLITES: IMPORTANT MEDIATORS IN THE CROSSTALK BETWEEN THE MICROBIOTA AND ADAPTIVE IMMUNITY

Short-chain fatty acids (SCFAs), tryptophan metabolites and bile salts are the principal metabolites produced by the gut microbiota which exert a protective effect against infections [9, 10]. Butyrate, propionate and succinate are known to act on intestinal homoeostasis, on mucus secretion, but also on the various cells of the immune system. Among other effects, butyrate has anti-inflammatory and anti-microbial effects. This action is exerted via the G-coupled protein receptors (GPR) found on the epithelial cells and the macrophages [9]. *F prausnitzii* produces large quantities of butyrate, which may partly explain its anti-inflammatory effect. It inactivates NF-κB and thus suppresses synthesis of the pro-inflammatory cytokines IFN-γ, TNF-α, IL-1β, IL-8 by the enterocytes [8] (**Figure 3**) . It also induces metabolic and epigenetic modifications (via histone deacetylases, HDACs) macrophages in mice, thus amplifying their anti-microbial activities *in vitro* and *in vivo* [11]. Commensal bacteria can also metabolise tryptophan and produce antimicrobial substances. An example is the *Lactobacilli*, which utilise it as an energy source to synthesise an indole that binds to aryl hydrocarbon receptors (AhR) present on the ILC3. AhR triggers IL-22 secretion by the ILCs and this further drives the secretion of AMPs and protects against infections [9].

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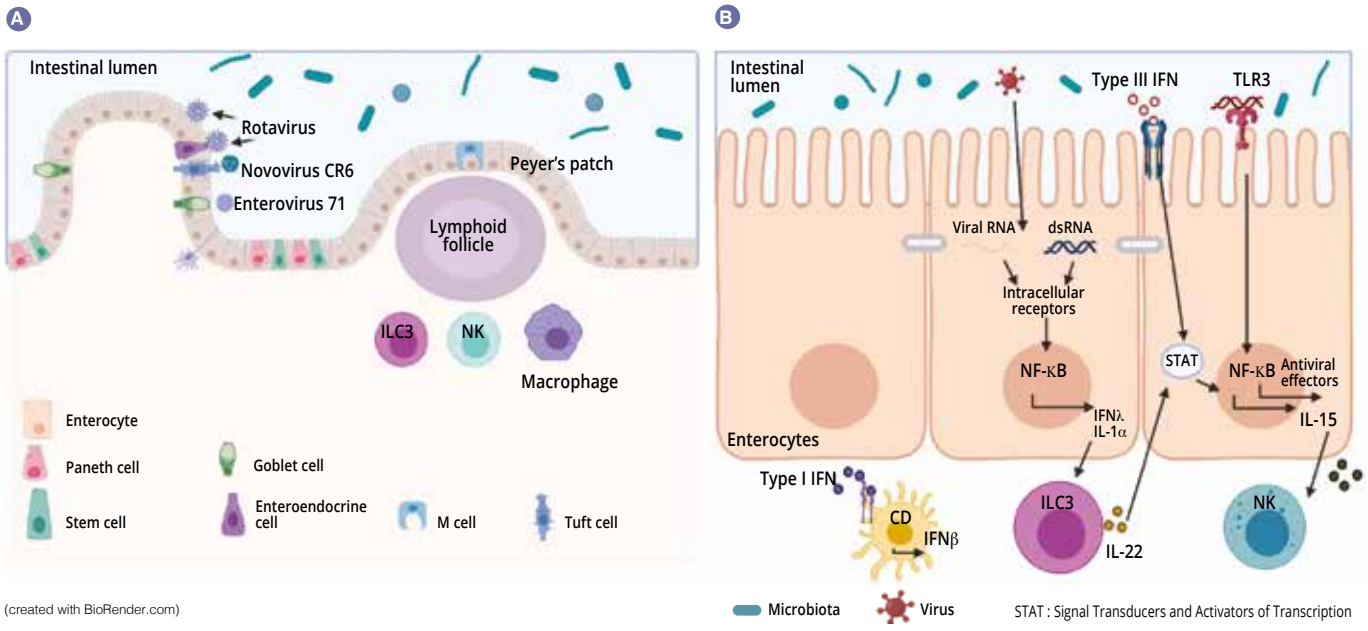
▼FIGURE 4

**A: Various cell types for enteric virus adhesion, B: Antiviral responses in the intestinal epithelial cells in case of infection**

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## MICROBIOTA – INTESTINAL IMMUNE SYSTEM CROSSTALK FOR PROTECTION AGAINST VIRAL INFECTIONS

Among the enteric viruses, norovirus and rotavirus are the main causes of gastroenteritis [12]. The enteric viruses infect various cell types: enterovirus 71 specifically infect the goblet cells, whereas the rotavirus has a preferential tropism for the enterocytes [13] (**Figure 4A**). The gut microbiota acts as a barrier against enteric viral infections. The viruses have evolved and become adapted to their host, implementing mechanisms that enable them to cross the intestinal barrier and escape barrier immunity: it is in fact difficult to infect mice effectively with human enteric viruses by the oral route [13]. Virus penetration into the enterocyte triggers the secretion of type III interferon (IFN). Detection of a virus can induce IL-lα, which activates the ILC3 to produce IL-22. This IL protects against enteric viral infections and acts synergistically with type III IFN to induce the expression of antiviral effectors and IL-15. Recognition of a virus by TLR-3 leads to the activation of the NF-κB pathway and to the production of IL-15 also. IL-15 activates the cytotoxic lymphocytes (NK cells). Those viruses, which have traversed the intestinal barrier, trigger the production of type I IFN by the macrophages of the lamina propria (**Figure 4B**). Some enteric viruses (rotavirus, reovirus, enterovirus) are able to adhere to the intestinal bacteria, enhancing penetration into the intestinal epithelial cells [13]. The SFB, which accelerate epithelial cell turnover produce protection against rotavirus infection in mice by expulsing infected cells [14]. The bile acids metabolised by the gut microbiota also act to protect the small intestine (but not the colon) from acute infection by norovirus in mice by enhancing the production of type III IFN in the small intestine [15].

## CONCLUSION

**The study of the relationship between the gut microbiota and intestinal immune response represents significant progress in gastroenterology research. Intestinal homeostasis is maintained due to the recognition of commensal bacteria by the cells of the innate system and the cells of the intestinal epithelium, either by direct contact (in the case of SFB), or *via* the synthesis of metabolites by the microbiota. The loss of homeostasis (intestinal dysbiosis, infections etc.) causes stimulation of the innate responses and an activation of the adaptive system. Poor "management" of inflammation can result in the onset of disease, such as post infectious irritable bowel syndrome.**


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# MICROBIOTA GUT-BRAIN AXIS IN IRRITABLE BOWEL SYNDROME

**By Prof. Premysl Bercik**
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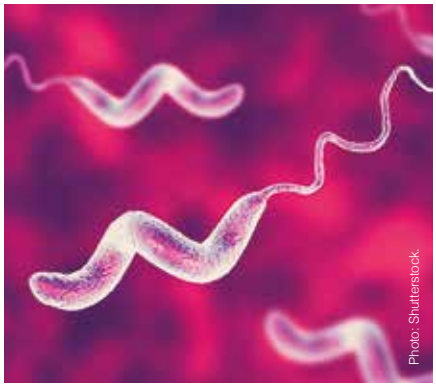
**Irritable bowel syndrome (IBS), characterized by abdominal pain and altered bowel habits, is the most common functional gastrointestinal disorder and is frequently accompanied by psychiatric comorbidities. Its pathophysiology is not fully understood but impairment in the gut-brain communication seems to underlie its genesis, with microbiota playing an important role in this process. Microbiota composition and its metabolic activity differ between patients with IBS and healthy controls, but no specific profiles have been identified. However, transplantation of fecal microbiota from IBS patients into germ-free mice induces gut dysfunction, immune activation and altered behavior in the murine host, similar to those observed in patients, thus suggesting its causal role. Furthermore, treatment with antibiotics or probiotics improve symptoms in some patients with IBS. Better understanding of the microbial-host interactions that lead to gut symptoms and psychiatric comorbidities, as well as discovery of new biomarkers that identify those who may benefit from microbiota directed treatments, are needed for optimized management of patients with IBS.**



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## IRRITABLE BOWEL SYNDROME

Irritable bowel syndrome (IBS) is a functional gastrointestinal disorder characterized by recurrent abdominal pain, that is associated with changes in stool frequency or stool form, in the absence of any organic disorder. Using ROME IV criteria, IBS is classified into four subtypes: IBS with predominant constipation (IBS-C), IBS with predominant diarrhea (IBS-D), with mixed bowel habits (IBS-M) or IBS, unsubtyped (IBS-U) which does not meet the criteria for IBS-C, D, or M [1]. Psychiatric comorbidities, such as anxiety, depression and somatization are common in patients with IBS (**Figure 1**).

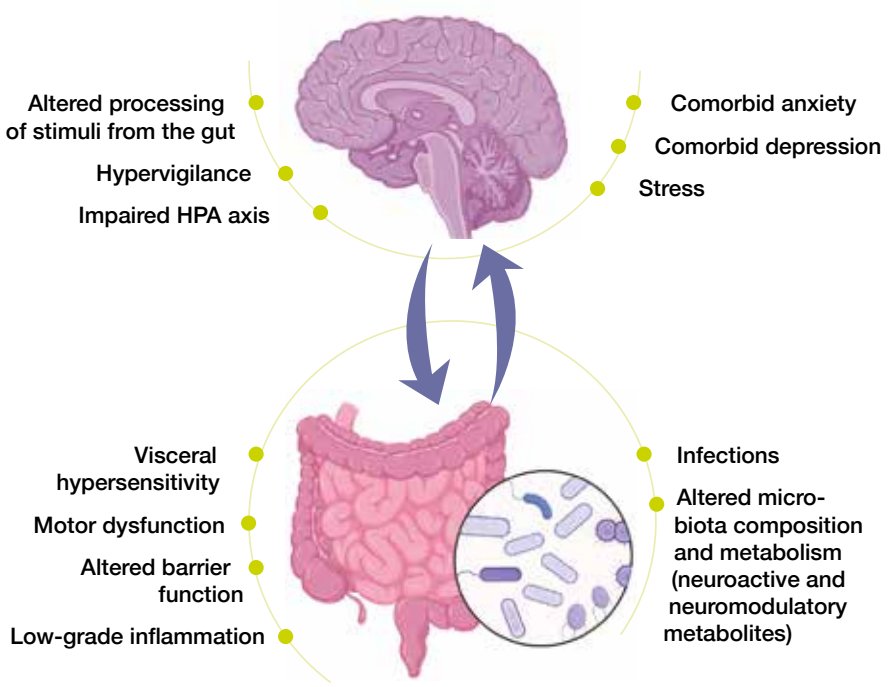
Although IBS prevalence rates appear to differ between countries, it is estimated to affect around 1 in 10 people globally [2]. IBS can develop at any age, but its onset is often usually between age of 20 and 30. Women are almost twice as likely as men to have symptoms of IBS, they also report to feel more fatigue and psychia-

tric comorbidities. The quality of life of IBS patients is severely affected, interfering with their everyday life, frequently resulting in missing work or school. The economic burden of IBS on healthcare systems and society is significant, with both direct and indirect costs. Mean annual direct cost for IBS patients was calculated at 1363 Euros, in addition to patients missing on average 8-22 days of their work per year.

Pathophysiology of IBS is not fully understood, but in general it stems from impaired gut-brain axis, a bidirectional communication between the digestive tract and the central nervous system. It likely involves multiple underlying mechanisms, including peripheral factors, such as visceral hypersensitivity, altered motility, increased intestinal permeability and low-grade inflammation. Among central factors, altered processing of signals from the gut, hypervigilance, stress, as well as psychiatric comorbidities, such as anxiety and depression, seem to play an important role. During the last decade, increasing attention has been given to gut microbiota as a key player in IBS.

▼FIGURE 1

**IBS: a bidirectional altered communication between the gut and the brain**




- IBS is characterized by abdominal pain and altered bowel habits.
- Its prevalence is around 11%, predominantly affecting women, it has a significant socio-economic impact.
- Its pathophysiology is not fully understood, it is considered to be a disorder of the gut-brain interaction.

## MICROBIOME IN IRRITABLE BOWEL SYNDROME

There are several lines of evidence, both from clinical studies and animal models, that implicate gut microbiota in IBS. First, bacterial gastroenteritis is the strongest risk factor for IBS, with 11-14% of patients developing chronic symptoms after acute infection with *Campylobacter, Salmonella, Shigella, Escherichia coli* or *Clostridioides difficile* infection [3]. Clinical data suggest that female sex, younger age, severity of infection and preceding psychiatric morbidity are risk factors for IBS. In addition, variants in genes related to the gut permeability, recognition of bacteria and innate immune responses have been identified.

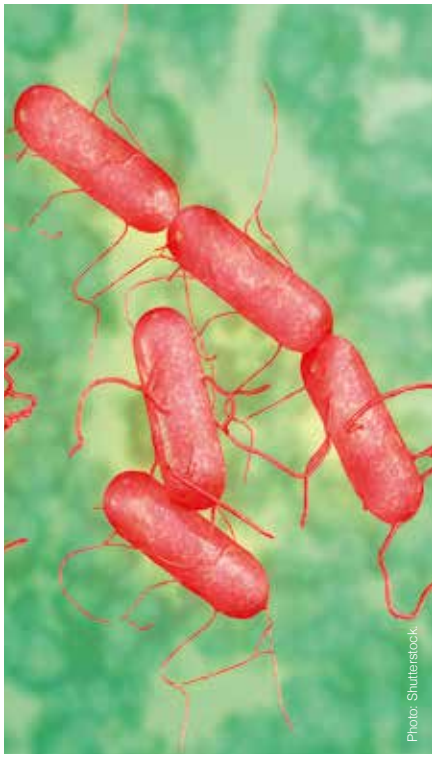
Second line of evidence comes from clinical studies that demonstrated that certain antibiotics may improve symptoms in a proportion of patients with IBS [4]. On the other hand, clinical data also suggest that use of antibiotics, with likely subsequent intestinal dysbiosis, can lead to symptoms generation. And finally, multiple clinical trials have suggested that specific probiotics improve symptoms of IBS, such as abdominal pain, diarrhea or bloating.

The bacterial population thriving in the gut, collectively termed the gut microbiota is one of the major determinants of gut homeostasis. Accumulating data show that gut microbial composition and its metabolic activity differ between IBS patients and healthy controls, and that they associate with intestinal symptoms, as well as with anxiety and depression. However, the results from individual studies are highly variable and there seems to be no unique microbial profile that could be attributed to IBS. Despite this, a recent meta-analysis identified several microbial features, including increase in family *Enterobacteriaceae*, family *Lactobacillaceae*, and genus *Bacteroides* and decrease in uncultured *Clostridiales*, genus *Faecalibacterium*, and genus *Bifidobacterium* in patients with IBS compared to healthy controls (**Figure 2**) [5]. There are also multiple bacterial or host-microbial metabolites that are altered in patient with IBS, including phosphatidylcholine, dopamine, p-hydroxybenzoic acid, bile acids, tryptamine and histamine metabolites. However, all these findings are suggestive of association but not of causation.

The microbiota humanized mouse model is a valuable tool to establish the causal role of the gut microbiota in the IBS, and to study the underlying mechanisms leading to gut dysfunction. We used stool microbiota from patients with IBS-D and from age- and sex-matched healthy controls to colonize germ-free mice and studied them 4 weeks later. Mice colonized with IBS-D microbiota developed faster gastrointestinal transit, changes in gut barrier function and low-grade intestinal inflammation, compared to mice colonized with microbiota from healthy controls [6]. Furthermore, mice that were colonized with microbiota from patients with comorbid anxiety also developed anxiety-like behavior, suggesting that microbiome transplantation from IBS patients into the murine host not only alters the gut function, but also impairs the gut-brain communication. These functional ab-

normalities were associated with changes in multiple neuro-immune gene networks, as well as changes in many microbial and host metabolites. Interestingly, treatment with a probiotic normalized gastrointestinal transit and anxiety-like behavior in mice with IBS-D microbiota, which was associated with changes in microbiota profiles and bacterial indole production, reaffirming the notion that the gut microbiome plays a key role in the gut-brain communication [7].

- Bacterial gastroenteritis is the most significant risk factor for IBS.
- Microbiota-directed treatment (antibiotics, probiotics) can improve IBS symptoms.
- Microbiota profiles and metabolism differ in patients with IBS and healthy controls.
- Microbiota transplantation from IBS patients into germ-free mice can induce gut and brain dysfunction.



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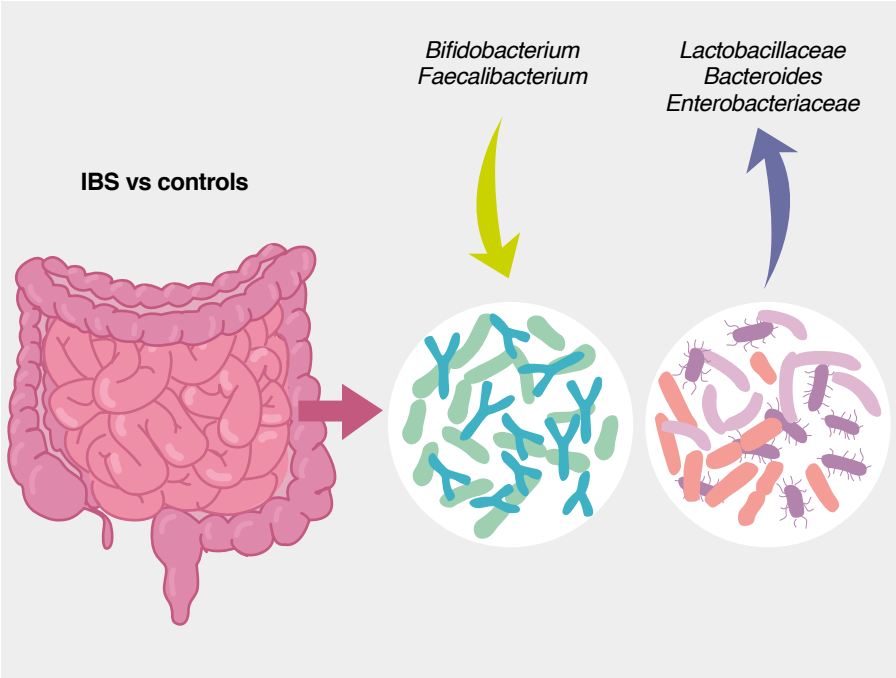
▼FIGURE 2

**Gut microbiota in IBS patients.**




## MICROBIOTA-GUT-BRAIN AXIS

The gut-brain axis is a bidirectional communication system between the gut and the brain integrated via neural, hormonal, and immunological signalling. Growing evidence suggests that the gut microbiota plays a key role in the communication between the gastrointestinal tract and the central nervous system, with most data being obtained from animal studies [8]. Germ-free mice have abnormal behavior, associated with changes in expression of multiple genes and chemistry in the brain, altered blood-brain barrier, changes in morphology of brain regions involved in control of mood and anxiety (amygdala and hippocampus), altered myelination profile and plasticity, as well as global defects in brain microglia. Most of these abnormalities are normalized after bacterial colonization. Microbiota also modifies behavior in conventional mice, as administration of non-absorbable antimicrobials can increase their exploratory behavior, together with changes in Brain- Derived Neurotrophic Factor (BDNF) in the hippocampus and amygdala. Changes in behavior induced by antibiotics have been also described in patients treated for acute infections or during eradication of chronic *Helicobacter pylori* infection; this condition was coined "antibiotic-induced psychosis". Interestingly, a recent large population-based study found that use of antibiotics in early childhood was associated with an increased risk of developing mental health disorders in later life.

However, the most obvious case for the microbiota-gut-brain axis comes from patients with cirrhosis-associated hepatic encephalopathy that manifest with changes in behavior, mood and cognition [9]. These patients show dramatic improvement in brain function after administration of antibiotics or laxatives, and recent studies suggested that similar amelioration can be also achieved by fecal microbiota transplantation.

During recent years, multiple studies investigated gut microbiome in patients with psychiatric disorders, such as major depression and generalized anxiety, and found that the microbial profiles differed between patients and healthy controls. Furthermore, transferring microbiota from patients into germ-free or antibiotic treated rodents induced anxiety and depressive-like beha-

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viors. This raises question whether those probiotics, which showed beneficial effects on behavior and brain chemistry in animal models, could be used to treat patients with psychiatric diseases. The results of the few studies completed so far suggest that probiotics, if used as an adjunctive treatment, might improve symptoms in some patients with major depressive disorder [10].

We conducted a pilot RCT study in patients with IBS and comorbid depression to assess effects of a probiotic that showed beneficial effects on behavior and brain chemistry in several mouse models [11]. We found that compared to placebo, a 6-week probiotic treatment improved depression scores and overall symptoms of IBS. This was associated with changes in neuronal activation in the amygdala and other brain regions involved in mood control, as assessed by functional magnetic resonance imaging. This suggest that some probiotics may produce neuroactive metabolites that could be harnessed not only for treatment of patients with functional bowel disorders, but also for those with mental health issues. However, more rigorous clinical studies are needed to confirm and validate these findings.

- Gut microbiota modifies behavior, as well as brain chemistry and structure in animal models.
- Clinical data suggest that microbiome is involved in cognition and mood disorders, such as hepatic encephalopathy, major depression and generalized anxiety.
- Specific probiotics might improve depressive behavior in patients, but more clinical data are needed to confirm these findings.

## CONCLUSION

**Irritable bowel syndrome is a common functional gastrointestinal disorder with frequent psychiatric comorbidities, that negatively affects patients quality of life and has significant socio-economic impact. Its pathophysiology is not fully understood, but it is likely multifactorial and is considered to be a disorder of the gut-brain interaction. Gut microbiota appears to play a key role in IBS, possibly through interactions with the immune or neural system, although the exact underlying mechanisms have to be clarified. Gut bacteria have the capacity to affect behavior and brain structure, and some probiotics might be beneficial for treatment of both gut and brain dysfunction.**


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# PLAUSIBILITY OF A PATHOPHYSIOLOGICAL ROLE FOR ALTERED GUT MICROBIOTA IN THE IRRITABLE BOWEL SYNDROME

**The irritable bowel syndrome (IBS) is a common functional bowel disorder characterized by abdominal pain, which is associated with changes in stool frequency and/or stool consistency. While not established yet, the pathogenesis and a multitude of putative pathophysiological mechanisms have been proposed, including: disordered motility, visceral hypersensitivity, low-grade inflammation, altered microbiota, immune activation, adverse reactions to foods and central nervous system dysfunction, etc. In 2017, five putative criteria for mechanisms in functional gastrointestinal disorders were published in *Gut*. Here we discuss to which extent altered gut microbiota fulfills these plausibility criteria in the context of IBS and review the available literature on the subject.**

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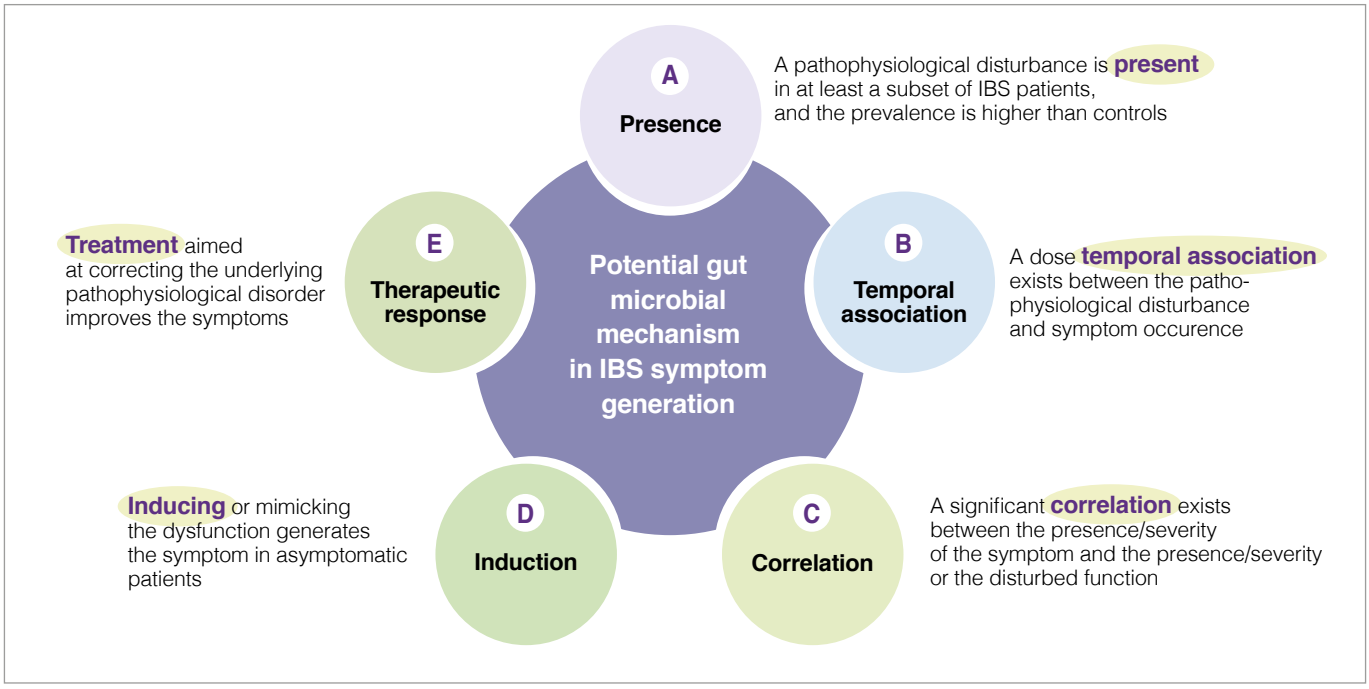
## INTRODUCTION

The most common functional bowel disorder, the irritable bowel syndrome (IBS), is characterized by abdominal pain or discomfort and is associated with changes in stool frequency and/or consistency, without identifiable structural or biochemical abnormalities indicating organic disease during routine investigations [1, 2]. Besides abdominal pain, patients also report other gastrointestinal symptoms as bloating, abdominal distention, and flatulence. IBS can be divided into different subtypes, based on the most dominant stool consistency: IBS-C (predominant constipation), IBS-D (predominant diarrhea), and IBS-M (IBS with mixed bowel habits). In terms of pathophysiology, IBS is considered a heterogeneous disorder and different mechanisms have been implicated, including gastrointestinal dysmotility, visceral

▼FIGURE 1

**Plausibility criteria for pathophysiological mechanisms in IBS disorders based on a consensus publication [6], as can be applied for the role of gut microbial mechanisms in the pathogenesis of IBS symptoms.**




hypersensitivity, dysfunction of the brain-gut axis and, more recently, changes in bile salt composition and handling, low-grade inflammation, mucosal immune activation, and altered intestinal microbiota [3].

The last decade has seen a major surge in interest in the role of gut microbiota in IBS. The microbial community of the gut exerts a number of functions, including the metabolism of indigestible polysaccharides, the absorption of certain nutrients and ions, the uptake and deposition of dietary lipids, regulation of bile acid metabolism, and the production of vitamins such as folate, biotin and vitamin K [3, 4]. By competing with microbial pathogens, it reinforces the gastrointestinal barrier protection. While interacting intensely with the mucosa, the gut microbiota also affect the immune system and gut-brain signaling of the host [5]. These diverse properties identify gut microbiota as a potential major contributor to the pathophysiology and as an attractive target for therapy in IBS.

Indeed, multiple mechanisms associated with the gut microbial ecosystem, have been identified in IBS pathophysiological studies. They have led to variable arguments and observations to support the relevance of these individual candidate mechanisms. To advance the field there is a need to identify the level of relevance of such putative pathophysiological processes, as this would enhance the knowledge and may prioritize targets for therapeutic innovation or optimization. A few years ago, a group of international experts developed five plausibility criteria for mechanisms in functional gastrointestinal disorders such as IBS [6]. They are based on aspects such as presence, temporal association, correlation between level of impairment and symptom severity, induction in healthy subjects and treatment response (or congruent natural history if no treatment is possible) (**Figure 1**). The following sections will evaluate the putative hypothesis that implicate a change in gut microbiota as a mechanism in IBS symptom generation and presentation (Box). The current knowledge regarding gut microbiota in IBS is summarized, and areas for further research are identified.

## PLAUSIBILITY OF A PATHOPHYSIOLOGICAL ROLE FOR GUT MICROBIOTA IN IBS

### PRESENCE OF ALTERED GUT MICROBIOTA IN IBS (A)

The first plausibility criterion is that changes in gut microbiota are found in at least a subset of IBS patients [6]. Several studies have investigated the presence and type of alterations of gut microbiota in IBS compared to healthy controls. Pittayanon and colleagues have published in a 2019 a systematic review of 24 studies from 22 publications comparing gut microbiota of patients with IBS (mainly adult) with microbiota of healthy individuals [7]. They concluded that family *Enterobacteriaceae*, family *Lactobacillaceae* and genus *Bacteroides* were increased, whereas *Clostridiales* I, genus *Faecalibacterium*, and genus *Bifidobacterium* were decreased in patients with IBS compared with controls [7]. While these observations make a case for altered microbiota in IBS, there is major heterogeneity in findings between different studies, sample sizes are usually small and most studies occurred in specialized care. Moreover, many studies did not correct statistics for multiple testing and did not consider dietary factors and prior pro- or antibiotic use. Also, no consistent differences were found between IBS stool subtypes [7]. The proportion of IBS patients in whom an altered gut microbiota composition can be identified remains unclear.

### TEMPORAL ASSOCIATION, OF ALTERED GUT MICROBIOTA WITH IBS SYMPTOMS (B)

The best evidence for a temporal association between changes in gut microbiota and IBS symptoms can be derived from the clinical entity of post-infection (PI-)IBS [8]. Approximately 10% of patients with infectious enteritis develop PI-IBS with female sex, younger age, psychological distress at the time of the gastroenteritis, and se-

verity of the acute infection as risk factors. Development of PI-IBS is associated with changes in the intestinal microbiome, as well as mucosal alterations (low-grade inflammation, entero-endocrine cell hyperplasia) [8]. However, the changes in microbiota in PI-IBS seem to differ from those described in IBS patients in general.

## CORRELATION BETWEEN LEVEL OF CHANGE OF GUT MICROBIOTA AND IBS SYMPTOM SEVERITY (C)

Very few studies have tried to correlate IBS symptom severity with the degree of change in gut microbiota composition, also referred to as "dysbiosis". Most of them failed to identify significant correlations between differences in fecal microbiota abundance or composition and IBS symptom severity [7, 9]. In a large IBS patient dataset, the Gothenburg group used machine learning to identify an intestinal microbial signature that is able to predict IBS symptom severity [9], hinting at a quantitative relationship between gut microbiota alterations and IBS severity. However, confirmation is needed from other studies, and perhaps these should include non-tertiary care patient samples, where the variation in symptom severity may be larger.

## INDUCTION OF IBS SYMPTOMS IN HEALTHY SUBJECTS THROUGH CHANGES IN GUT MICROBIOTA (D)

The fourth plausibility criterion, as described in the initial manuscript [6], is one of the most difficult to fulfill. There are very few suitable data for the different candidate pathophysiological mechanisms, and this also applies to gut microbiota alterations as a mechanism. The most supportive observation is probably derived from development of IBS after treatment of a non-gastrointestinal infection with systemic antibiotics [10]. The nature of the disturbance of gut microbiota after antibiotics, and the degree of similarity with gut microbiota in IBS are still unknown.

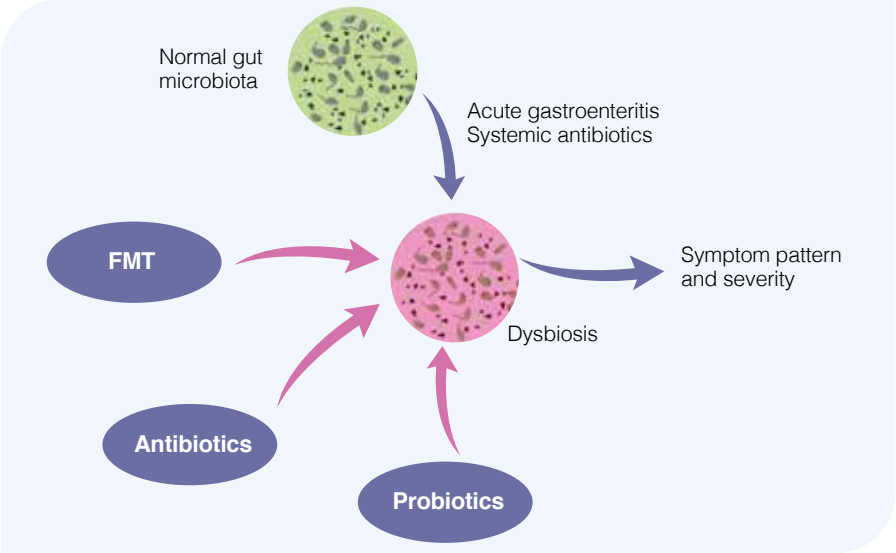
## RESPONSE TO TREATMENT THAT TARGETS GUT MICROBIOTA COMPOSITION (E)

This section is the most extensively studied one when considering plausibility criteria for altered gut microbiota composition as a pathophysiological mechanism in IBS.

▼FIGURE 2

**Pathophysiological relevance of changes in gut microbiota in irritable bowel syndrome.**

Normal gut microbiota composition reflects the state of health, without IBS symptoms. Acute events, such as an acute gastroenteritis or intake of systemic antibiotics may alter gut microbiota composition, leading to IBS symptoms. This may be therapeutically corrected by the use of non-absorbable antibiotics, probiotics or fecal microbiota transfer.




One line of evidence is the beneficial therapeutic effect of poorly absorbable antibiotics, clearly targeting gut microbiota [11, 12]. Two studies with neomycin and five trials with rifaximin showed efficacy of these poorly absorbable broad spectrum in non-constipated IBS patients [11-14]. In addition, a trial evaluating the safety and efficacy of repeat treatment with rifaximin confirmed as well the feasibility of this therapy upon symptom recurrence [15].

Probiotics are defined as preparations with living micro-organisms that confer a health benefit to the host when administered in adequate amounts. Several meta-analysis confirmed the efficacy of probiotics, as a group, to improve symptoms of IBS [11, 16]. However, the heterogeneity of study designs and endpoints, and the relative paucity of studies with specific probiotic types preclude making strong conclusion at the level of individual preparations. In contrast, prebiotics, substrates that are selectively utilized by host microorganisms conferring a health benefit to the host, showed no efficacy in improving IBS symptoms based on recent meta-analyses [11, 17].

Fecal microbiota transplantation (FMT) is probably the most direct way of targeting the gut microbiota for symptom control in IBS [18]. Studies to date have yielded highly variable outcomes, from no effect to symptomatic benefit, but also worsening of symptoms, generating conflicting conclusions in meta-analyses [19, 20]. However, recent studies have shown FMT-induced changes in gut microbiota composition associated with (transient) symptomatic benefit, and have implicated donor selection as a critical issue [21, 22].

# UNSOLVED ISSUES AND FUTURE STUDIES

Taken together, changes in gut microbiota composition seem to fulfill the plausibility criteria for pathophysiological relevance in the irritable bowel syndrome [6]. The findings are summarized (**Figure 2**). However, there is a clear need for additional knowledge and research. More quantitative and better controlled studies characterizing the gut microbiota in IBS and controls are needed, and these should preferably include large patient cohorts also from primary care. This will allow a better understanding of the changes in gut microbiota in IBS at all levels of care, and has the potential to confirm a correlation between the magnitude of changes in gut microbiota composition and IBS symptom severity. In addition, longitudinal studies in

▼TABLE 1

**Highlight box: Summary of fulfillment of plausibility criteria for altered gut microbiota in IBS.**

| CRITERION | EVIDENCE | LEVEL OF EVIDENCE | REFERENCES |
|---|---|---|---|
| **A** **Presence** | A systematic review summarized the literature on significant differences in gut microbiota in patients with IBS compared with controls | Several papers reporting differences in gut microbiota composition in IBS versus health. Summarized in a recent meta-analysis (Level 5) | [7] |
| **B** **Temporal association** | The best evidence is found in the clinical entity of post-infection IBS | Several papers documenting increased occurrence of IBS after an acute (bacterial) gastro-enteritis. Summarized in the 2019 Rome Working team paper (Level 5) | [8] |
| **C** **Correlation** | An intestinal microbial signature associated with IBS symptom severity has been described | Limited data so far: only one report claiming a correlation of microbiota profile with IBS severity (Level 2) | [9] |
| **D** **Induction** | There is a paucity of data on this aspect. One supportive observation is the onset of IBS after systemic antibiotic intake | No data supporting this. Only the reported triggering by antibiotics in one paper (Level 1) | [10] |
| **E** **Therapeutic response** | This aspect is supported by beneficial therapeutic effects of poorly absorbable antibiotics, probiotics and fecal microbiota transplantation in IBS | Several studies in the literature reporting beneficial effects of microbiota-targeting therapeutic interventions in IBS. Some supported by meta-analysis (Level 5) | [11-22] |

IBS will be needed to further establish the temporal relationship between gut microbiota changes and symptom pattern and severity over time, in or outside the frame of a treatment trial.

There is a continued need for higher quality probiotic trials in IBS, using appropriate treatment lengths and validated endpoints, similar to those with pharmacological agents. Finally many new data on the use of FMT in IBS are expected, with a potential to clarify the best modalities and the efficacy of this treatment option.

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# NEW PERSPECTIVES IN AUTISM: THE ROLE OF MICROBIOTA IN SOCIAL COMMUNICATION

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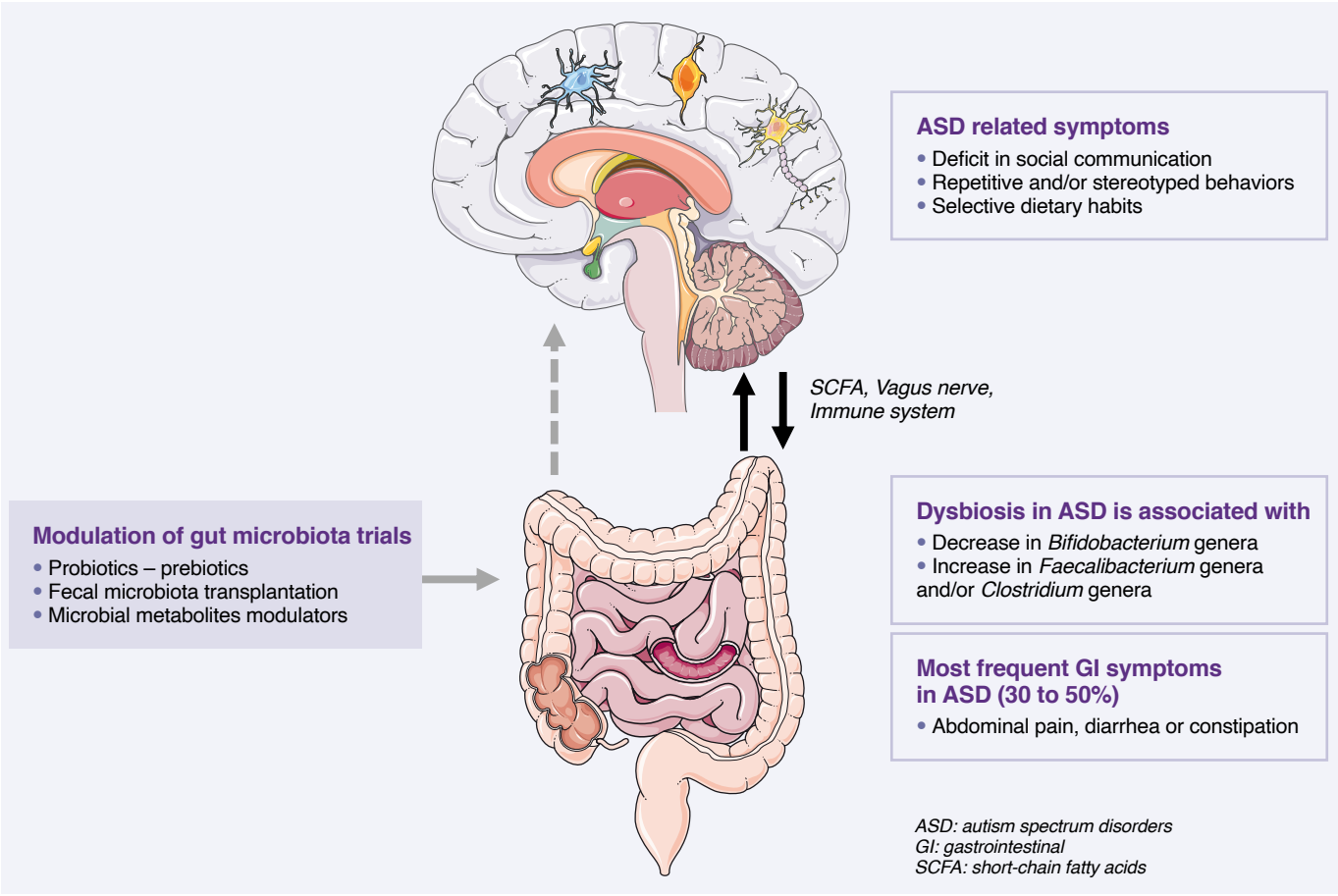
**Autism Spectrum Disorders (ASD) are complex neurodevelopmental disorders affecting 1% of the general population and characterized by a deficit in social communication and repetitive/stereotyped behaviors. The pathophysiological mechanisms behind ASD are still poorly understood [1]. Thirty to 50% of individuals with ASD present gastrointestinal (GI) symptoms such as abdominal pain, diarrhea, constipation, which affect their quality of life and their global functioning. Interestingly, the occurrence and the severity of GI symptoms are strongly correlated with autistic symptoms [2]. Whereas the etiology of GI symptoms are still unknown, several studies suggested that ASD could result from an imbalance in the gut microbiota (GM) composition [3]. Consistent findings suggest robust interactions between GM and the central nervous system (CNS). GM directly affects neurodevelopment by impacting neurogenesis, neuron survival, brain growth and myelination. The modulation of GM using pro- or prebiotics or fecal microbiota transplantation (FMT) in individuals with ASD shows beneficial and long-term effects on GI symptoms and core autistic symptoms. Larger double-blind randomized trials are however needed to confirm the efficacy of microbial-based therapies in ASD, specifically at an early and critical stage of neurodevelopment.**

## GUT MICROBIOTA, CENTRAL NERVOUS SYSTEM AND NEURODEVELOPMENT

The human gut microbiota (GM) consists of approximately $10^{13}$ microorganisms, mainly bacteria, fungi and viruses. GM plays a central role in human health, ensuring intestinal barrier function, modulation of the immune response and metabolic synthesis, but also a direct protection against infections. Dysbiosis characterized by an unbalanced GM has been associated with several diseases such as inflammatory bowel diseases, cancers, diabetes or obesity [4]. Consistent findings also suggest robust interactions between GM and the central nervous system (CNS) [5] (**Figure 1**).

▼ FIGURE 1

**The relationship between gut microbiota-brain axis and autism spectrum disorders and the therapeutic strategies tested to modulate it.**




A reciprocal crosstalk between brain and GM is mediated by microbial metabolites (mainly short chain fatty acids) and immune modulators directly by crossing both the blood-gut and the blood-brain barriers and indirectly *via* the stimulation of the vagus nerve [6]. Evidence supports the involvement of GM in the regulation of both human behaviors and cognitions - specifically socio-communication skills – even if its exact mechanisms are still unknown [5].

ASD are neurodevelopmental disorders characterized by impairments in social communication, social interactions, and repetitive/stereotyped behaviors, of childhood onset affecting approximately 1% of the general population. The determinism of ASD is mainly driven by genetic factors, with a heritability estimated reaching 0.8-0.9, but pre- and post-natal environmental events may act as precipitating factors or modulators of the symptom severity. The trajectory of brain development at an early stage of life overlaps with those of the GM. This latter begins to develop early after birth and its composition stabilizes into an adult-like profile around the age of 3 years old. GM early composition is deeply influenced by environmental factors such as the place of birth, delivery mode, breastfeeding and xenobiotics (*e.g.* antibiotics use).

Germ-free mice are a model lacking all microorganisms *e.g* are microbiologically axenic (no living organisms can be cultured from germ-free mouse specimens). Germ-free mouse models are valuable to decipher the mechanisms underlying the roles of GM in neurodevelopment, but also the relationship between microbiome and disease. Studies showed that germ-free mice exhibit i) default in brain-blood barrier permeability; ii) higher brain volume; iii) more immature microglia gene expression and less microglial immune responsiveness; iv) increased myelination ; and v) decreased Brain-Derived Neurotrophic Factor expression and in a subunit of N-Methyl-D-Aspartate receptors [5, 7]. All these data stress the role of GM in blood-brain barrier formation and integrity, neurogenesis, microglia homeostasis, myelination and brain growth/function.

## GUT MICROBIOTA, GASTRO-INTESTINAL SYMPTOMS AND AUTISM SPECTRUM DISORDERS

Germ-free mice displayed autistic-like behaviors such as social avoidance, repetitive/stereotyped behaviors, lack of interest in social novelty. Some of these behaviors disappeared after colonization by a GM from wild type mice whereas colonization by GM from ASD mouse models increased these behaviors. GM seems indeed crucial for the programming and presentation of social skills and adaptive behaviors [8].

A growing number of evidence shows that GI symptoms are overrepresented in ASD children. GI symptoms such as abdominal pain, constipation, diarrhea are reported in nearly 30-50% of patients with ASD and profoundly impact the quality of life of children [1]. GI symptom severity was correlated with the severity of autistic symptoms and gut dysbiosis is well documented even if there is still no specific signature related to autistic symptoms. Studies exploring GM

reported differences in microbiota diversity, and abnormal metabolite patterns when compared to healthy controls. Two recent meta-analyses exploring GM composition in ASD patients reported a decrease in *Bifidobacterium* and increase in *Faecalibacterium* and *Clostridium* genera in ASD patients [9, 10] compared to controls. The exploration of the fecal metabolome also displayed an increase in p-cresol, a bacterial metabolite derived from tyrosine, in individuals with ASD. All together, these data may indicate the potential association between GM abnormalities and GI symptoms in ASD patients.

However, most studies have heterogeneous results and methodological limitations. Merely confounding factors such as different countries with different lifestyles and dietary habits are major drawbacks of these studies. Indeed, a recent study in a large cohort of 247 subjects with ASD did not report direct links between ASD diagnosis or autistic symptoms, and GM dysbiosis. Dysbiosis was associated with a less-diversified diet which is common in patients with ASD [8].

## MODULATION OF THE GUT MICROBIOTA IN AUTISM SPECTRUM DISORDERS

A growing number of studies explored the potential impact of microbiota-based therapeutic strategies to improve GI symptoms and core symptoms in individuals with ASD.

Probiotics, live microorganisms, have been used in ASD and could have a beneficial effect on patients with ASD. Some preclinical studies showed increased social interactions with probiotics supplementation (*Bacteroides fragilis*, *Lactobacillus reuteri*) in mouse models of ASD. The improvement of social communication was linked with an increased oxytocin expression in CNS. In humans, several studies reported positive effects of probiotic treatments on GM composition and GI symptoms in ASD [11]. However, few of them reported an improvement of core autistic symptoms. Most clinical trials providing probiotics in autistic individuals showed inconsistency in terms of probiotics, dosage administration per day or in total, and duration of the whole treatment. Even if some studies suggest that probiotics could be interesting to prevent GI symptoms in ASD patients, the results request replication to guarantee the positive effect of such strategy.

**Autism Spectrum Disorders (ASD), Gastrointestinal (GI) symptoms & Gut Microbiota (GM)**

Almost 30-50% of patients with ASD reported GI symptoms such as abdominal pain, diarrhea, constipation. GM dysbiosis in ASD patients have been well explored and confirmed through meta-analysis even if there is no microbiome specific signature [9, 10]. The link between GM dysbiosis and ASD is still poorly understood. Some studies suggest direct links through the GM-brain axis influencing autistic symptoms and GI symptoms. More recently a study suggests that GM dysbiosis in ASD patients is mostly linked with the restrictive diet which is frequent in ASD patients [8].

Similarly, the efficacy of prebiotics, such as galacto-oligosaccharide (GOS) or fructo-oligosaccharide have been explored in ASD [12]. Chronically stressed mice showed alteration in GM and a decrease in social interest. Using this mouse model, the administration of prebiotics was associated with increased social interactions in these mice. In humans, the use of GOS associated with a casein-free and gluten-free diet showed improvement in GI symptoms and social interactions together with an increase in GM *Bifidobacterium* abundance. Appropriate double-blind randomized clinical studies are needed to confirm preliminary evidence.

Fecal microbiota transplantation (FMT) has also been studied in ASD. FMT involves transplanting GM from a donor to modify the GM of the receiver. Its efficacy on *Clostridioides difficile* infection is now well demonstrated, even in children. A recent exploratory unblinded and non-randomized clinical trial involving 18 children diagnosed with ASD and GI evaluated the effect of microbiota transfer therapy (MTT) - a modified FMT protocol [13]. MTT consisted in a two-week antibiotic treatment, a bowel cleansing, before receiving the MTT treatment which consisted of a high dose through oral or rectal administration followed by an oral maintenance dose for 7-8 weeks. Adverse events at the initiation of vancomycin treatment were observed (disruptive behaviors, hyperkinesia) but disappeared spontaneously after 3 days of treatment. The MTT protocol led to a significant improvement in GI symptoms after the following survey of 8 weeks. More surprisingly, an improvement on core autistic symptoms (stereotyped and repetitive behaviors, social communication skills) had also been observed 8 weeks after MTT. Interestingly, improvement on GI symptoms and autistic symptoms persisted 2 years after treatment and was correlated with GM increased diversity [14]. Two years after MTT, the average reduction of the Gastrointestinal Symptom Rating Scale (GSRS) total score was still over 50%. Changes in autistic symptoms measured with the Childhood Autism Rating Scale - CARS, the Social Responsiveness Scale - SRS, or the Autistic Behavior Checklist - ABC were all positively correlated with percent changes in GSRS scores. These results are not yet confirmed by placebo-controlled double-blind randomized studies.

Recently, a pilot open label clinical trial in ASD has explored the effect of an oral GI-restricted adsorbent (AB-2004) modulating several GM metabolites. The authors reported a decrease in anxiety-like behaviors in mice, driven by a gut microbial metabolite decrease [15]. The study also presented preliminary results from a clinical trial in which an AB-2004 weight-adjusted dose was administered, for 8 weeks, to 30 adolescents with ASD. At week 8, reduced levels of GM metabolites in plasma and urine were observed. More interestingly, after treatment, less subjects displayed GI symptoms but also ASD-associated behaviors, anxiety, and irritability. There was also a remnant effect with a persistence of the efficacy at 4 weeks after treatment discontinuation [15]. The factors linking clinical improvements and

administration of AB-2004 remain to be determined, some indirect factors have not been studied such as the effect of AB-2004 on nutrition changes, immune status or GI function. Larger, double-blind placebo-controlled studies trials are warranted to further dissect the role of AB-2004 in social communication in humans.

In the context of the lack of specific treatment for GI symptoms and autistic symptoms in ASD patients, new well tolerated therapeutic strategies targeting GM or microbial metabolites such as FMT/MTT need to be more performed specifically in early and critical stages of brain development during childhood.

**Fecal Microbiota Transplantation (FMT) in ASD patients**

FMT is a new efficient way to modulate GM used in gastrointestinal and endocrine diseases. Fecal microbiota transplantation (FMT) could represent a new and efficient opportunity to modulate/reset GM in ASD. Using mouse models of ASD, FMT efficiently improved autistic like core symptoms such as social communication deficits and stereotyped behaviors. In humans, a pilot open-label trial in adults with ASD suggested that FMT could improve not only GI symptoms but also social reciprocity and stereotyped behaviors. There was a remnant effect with a persistent efficacy 2 years after treatment discontinuation. Interestingly the efficacy was associated with an increased GM diversity. FMT could be a new cutting-edge therapeutic strategy to treat GI and behavioral symptoms in ASD children, and merely open new avenues toward physiology of social communication in humans.

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## CONCLUSION

**The impact of gut microbiota on the neurodevelopmental trajectory of children remains largely unexplored. At this point, there are no studies exploring the impact of gut microbiota modulation on the early phase of child development, particularly on social communication skills. Preliminary studies in adults and children have shown that GM modulation leads to improvement in GI symptoms and autistic behaviors. Based on these data, we will conduct a double-blind clinical trial in younger children with ASD at a very early stage of their cognitive development (before age 6). We aim to understand whether FMT performed at a critical developmental period will result in significant long-term improvement in the child's developmental trajectory.**


**Sources**

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BMI-22.11 - 05/2022