WGO Handbook

on

Gut Microbiome

A Global Perspective

Editors: Professors Eamonn M M Quigley and Uday Chand Ghoshal

World Gastroenterology Organisation (WGO)
555 East Wells Street, Suite 1100
Milwaukee, WI 53202 USA
Tel: +1 (414) 918-9798 • Fax: +1 (414) 276-3349
Email: info@worldgastroenterology.org
Web: www.worldgastroenterology.org

Follow Us
@WorldGastroOrg

#WDHD2020

WGO Handbook on Gut Microbiome

A Global Perspective

Table of Contents

WGO Handbook on Gut Microbiome
A Global Perspective

Table of Contents

1.1 The Gut Microbiome – a Global Perspective

Eamonn M M Quigley, MD, FRCP, FACP, MACG, FRCPI, MWGO

Lynda K and David M Underwood Center for Digestive Disorders
Houston Methodist Hospital
Weill Cornell Medical College
Houston, Texas, USA

Uday Chand Ghoshal, MD

Professor, Department of Gastroenterology
Sanjay Gandhi Postgraduate Institute of Medical Sciences
Lucknow, India

Justin Wu, MD

Chief Operating Officer, CUHK Medical Centre
Faculty of Medicine, Chinese University of Hong Kong
Hong Kong, China

Henry Cohen, MD, FACG, AGAF, MWGO

Professor of Gastroenterology
Director ECHO Project Uruguay
Montevideo, Uruguay

Just a few decades ago, when some of us were embarking on our medical careers, the era of infectious disease was considered to be over and accounts of the ravages of bacterial, viral and other microbial infections were of historical interest only. The Black Death, the Spanish Flu, outbreaks of botulism and field hospital amputations for infected wounds became nothing more than interesting footnotes in our microbiology and medical textbooks. The war with infectious agents was over and we had won! New challenges could now be addressed – cancer, neurodegenerative diseases and arteriosclerosis demanded our attention.

How wrong we were! How fatally we underestimated the guile and genetic intelligence of potential pathogens. We failed to anticipate how the human immunodeficiency virus (HIV) could paralyze our immune systems, how international travel and migration could expose the world to Ebola, West Nile, Zika and Chikengunya viruses, as well as trypanosomiasis, and how a totally new group of coronaviruses (Severe Acute Respiratory Syndrome-related coronavirus or SARS-CoV/CoV1, Middle East Respiratory Syndrome-related coronavirus or MERS-CoV) and, now, Severe Acute Respiratory Syndrome-related virus 2 (SARS-CoV2) the cause of the current coronavirus disease (COVID-19) could bring the world to its knees. Microbiology is back on the front page and we now look to a specialty that we regarded as peripheral to medicine to save the planet. A fundamental lesson from all of the aforementioned outbreaks is that they are global issues. Despite the nationalistic bleating and xenophobic braying of some politicians, COVID-19 is a problem for us all and demands integrated, pan-national solutions. These outbreaks expose the limitations of public health systems and, perversely, seem to invigorate an anti-science subculture that seeks to permeate media and politics and dictate response to the pandemic.

The choice of the gut microbiome, by WGO, as the topic for the 2020 World Digestive Health Day could not be more appropriate and timelier. The explosion in our knowledge of microbiomes, permitted by evolving developments in microbial science, has the potential to reveal very basic aspects of microbiome-host interactions and lead to the development of new diagnostic and therapeutic avenues.

Trans-national studies of the gut microbiome have revealed how much we have to learn from each other. Dramatic differences in gut microbial population between urban Western populations and rural Africans provided one of the first hints of the primacy of diet in the development of individual microbiomes[1]. As we learn of the role of the gut microbiome in defining susceptibility to both infec-

1.1 The Gut Microbiome – a Global Perspective, continued.

tious and non-infectious disease, we may gain insights into one factor that contributes to global variations in disease prevalence. Could such variability relate to differences in microbial populations? Or, getting back to the pandemic which so consumes our attention; could gut or lung microbiota determine susceptibility to becoming ill following exposure to SARS-CoV2[2]?

The microbiome is a global issue and the gut microbiome is no exception in this regard. How we foster or harm this essential contributor to human health will be a major contributor to global health. Perinatal care, infant and child nutrition and patterns of antibiotic use are but some of the factors that fundamentally impact on the gut microbiome and, thereby, perturb homeostasis. Antibiotic resistance, a global crisis, may be largely transferred via the microbiome! We must collaborate, share successes and failures and, together, mine the gut microbiome for new strategies to confront the challenges that pathogens of the future may present.

References:

1. Yatsunenko T, Rey FE, Manary MJ, Trehan I, Dominguez-Bello MG, Contreras M, Magris M, Hidalgo G, Baldassano RN, Anokhin AP, Heath AC, Warner B, Reeder J, Kuczynski J, Caporaso JG, Lozupone CA, Lauber C, Clemente JC, Knights D, Knight R, Gordon JI. Human gut microbiome viewed across age and geography. Nature 2012;486:222-7.
2. Zuo T, Zhang F, Lui GCY, Yeoh YK, Li AYL, Zhan H, Wan Y, Chung A, Cheung CP, Chen N, Lai CKC, Chen Z, Tso EYK, Fung KSC, Chan V, Ling L, Joynt G, Hui DSC, Chan FKL, Chan PKS, Ng SC. Alterations in Gut Microbiota of Patients With COVID-19 During Time of Hospitalization. Gastroenterology. 2020 [epub ahead of print].

1.2 The Gut Microbiome – an Introduction

Eamonn M M Quigley, MD, FRCP, FACP, MACG, FRCPI, MWGO

Lynda K and David M Underwood Center for Digestive Disorders
Houston Methodist Hospital
Weill Cornell Medical College
Houston, Texas, USA

Uday Chand Ghoshal, MD, DNB, DM, FACG

Professor, Department of Gastroenterology
Sanjay Gandhi Postgraduate Institute of Medical Sciences
Lucknow, India

Correspondence:
Eamonn M M Quigley MD FACP MACG
Houston Methodist Gastroenterology Associates
6550 Fannin St, SM 1201
Houston
Texas 77030
USA
equigley@houstonmethodist.org

Basic Definitions

Strictly speaking the term *microbiome* refers to an entire habitat, including the microorganisms (bacteria, archaea, lower and higher eurkaryotes, and viruses), their genomes (i.e., genes), and the surrounding environmental conditions. This term is also used to refer to the collection of genomes from all micro-organisms in a given environment and can be readily confused with the term *metagenome* which relates to the genetic material present in an environmental sample, consisting of the genomes of many individual organisms. In its strictest terms, *microbiota* should refer to all the micro-organisms found in the environment. The terms microbiome and microbiota are often, however, used interchangeably, even in the microbiology literature.

The gut microbiome and its implications in health and disease have rapidly evolved over the last two decades to become one of the hottest areas of medical research. The advent and widespread application of, first, high-throughput sequencing technology and, more recently, metagenomics, metabolomics, meta-transcriptomics and other 'omics, have, not only facilitated the enumeration of the microbial species that inhabit the human gut, but also provided predictions of microbial properties and their potential impact on the host, as well as measurements of biologically active microbial products (via metabolomics, for example) (1). The real breakthrough came with the recognition that 16sRNA gene in bacterial genomes was highly conserved across all microorganisms yet varied between them; thereby becoming a valuable "fingerprint" for different bacteria. Using this approach, *high-throughput sequencing* could identify, admittedly only at a fairly high level of organization (phylum and genus, for example), the microbial composition of samples. *Metagenomics,* using shotgun sequencing, for example, can characterize bacteria in much more detail (down to species and strain) by extracting bacterial DNA directly from the sample and through various steps recreating the genome of the organism and also, thereby, providing predictions of function based on gene analysis. Finally, *metabolomics* assays actual metabolic products. Putting it simply high-throughput sequencing provides a good idea of what is there, metagenomics provides much more detail and tells us what they might do, and metabolomics and *meta-transcriptomics* identifies what they actually produce.

These technological developments have spawned a host of studies describing changes in the microbiome in disease states and prompted enthusiasm for a role for microbiome analysis in diagnosis, prognosis or treatment selection. Interesting though these observations may be, they remain, for the most part, associations and well documented examples of truly causative microbial signatures remain singularly rare (2). Microbiome science has also revealed the therapeutic potential of the microbiome. While some microbiome-modulating strategies have been used on an empiric basis for decades if not centuries, recent research has begun to identify the various mechanisms whereby interventions, such as prebiotics, probiotics and fecal microbiota transfer (FMT) might actually provide benefit (3).

1.2 The Gut Microbiome – an Introduction, continued.

The Gut Microbiome – the Basics

The nature and the importance of the complex interactions between the microbiome and its host are now well recognized and the contributions of this commensal relationship to the health of the host increasingly appreciated. Accordingly, one can predict how any disruption of this relationship might lead to pathological consequences for the host. Two well-defined clinical entities provide a vivid illustration of disrupted microbiome-host interactions: *Clostridioides difficile*-associated disease (CDAD) and *Helicobacter pylori* infection. The former serves as a dramatic reminder of the consequences of iatrogenic disruptions of a microbiome that, when intact, serves to protect us against pathogens and the latter exemplifies how host genome, bacterial properties and the immune response conspire to produce various disease phenotypes.

Many other studies have described associations between an altered microbiome and various gastrointestinal and systemic diseases and disorders. The term *"dysbiosis"* has been frequently used to refer to such apparently abnormal microbiota signatures and has entered the general lexicon in a manner that assumes clarity of definition. This term may have gained currency but it is far from satisfactory as it presumes that we know what constitutes a "normal" microbiome. While some common patterns have been described at a higher order level in between subjects in the general population in some studies (4), such is the variability between subjects and the impact of various personal and environmental factors at the levels of species and strain, that it seems premature to us the term "dysbiosis" to describe any human study. Furthermore, most human studies, regrettably, share one or more of the following limitations (3):

1. Such is the heterogeneity in bacterial populations between and within patient populations that it is still not possible to state with certainty what is normal in any given population.
2. Most studies are single point in time, rather than longitudinal, rendering it impossible to account for fluctuations in disease activity as well as the confounding impact of therapy. In other words, in can be nigh impossible to decide whether a given microbial signature represents state or trait in relation to a given disease. Only longitudinal studies with sampling at multiple time points can aid in making this distinction.
3. Diet, likely to be altered in many disease states, can significantly modify the microbiome (in both the long- and short-term) and has not been accounted for in many of the studies.
4. For obvious reasons of convenience, most human studies have been based on the analysis of fecal samples, an approach that disregards variations in bacterial populations along the length of the gastrointestinal tract and may fail to represent those bacterial populations that reside close to or adherent to the mucosa. Though more challenging in terms of access studies of the juxta-mucosal microbiome in the colon have revealed significant differences in health and disease it's microbial populations from those isolated from stool samples. Even more logistically challenging are studies of the small intestinal microbiome. Given that, for most nutrients, their digestion and absorption take place principally in the proximal small intestine (also the site of transport or absorption of most pharmaceuticals) and that the more distal small intestine is abundantly endowed in immune tissues, an understanding of the small intestinal microbiome is of critical importance.

What influences the microbiome? (Table 1)

Table 1 lists some of those factors which at this time seem most important in shaping the composition and thus the diversity of the gut microbiome. While their relative contributions continue to the subject of research and debate most would agree that age diet and diet are of critical importance, It is also worth noting that disease per se can alter microbiota composition – changes observed may be the consequence and not the cause of the disease.

1.2 The Gut Microbiome – an Introduction, continued.

Age	**Birth mode**
Infant feeding	**Diet**
Antibiotics	**Other medications**
Geography	**Disease**

Table 1. Some of the principal factors that influence the composition of the gut microbiome

Most authorities contend that the microbes that comprise the intestinal tract of infants are obtained during both the initial birthing process (vertical transmission) and from other humans and their environments early during infancy (horizontal transmission). However, more recently, using modern sequencing technology, this once accepted notion has been challenged by those proposing that neither the fetus, placenta, nor the amniotic fluid are sterile and are, in fact, the host of what is being termed the placental microbiome. This "in utero colonization hypothesis" states that the fetus is exposed to these microorganisms in utero where the colonization of the gastrointestinal tract begins (5). Although there is still no firm consensus on the prenatal microbiome (6), both hypotheses emphasize the importance of the first 1-3 years of life in the development of what will become the mature gut microbiome. Factors that most heavily influence its composition include method of delivery (vaginal vs caesarean section), source of nutrition (breast milk vs formula), maternal weight, prenatal diet, location of birth and exposure to antibiotics.

Over time, the composition of gut microbiota becomes more stable, with multiple members of *Bacteroidetes*, including those with butyrate-producing capacity, establishing a presence. It is well documented that the initial gut microbiome consists of bacteria that are able to metabolize lactose from either breast milk or cow's milk. As solid food is introduced into the diet, the microbiome evolves to one that can metabolize carbohydrates, proteins and fats and synthesize vitamins. By preadolescence (7 to 12 years of age), the number of bacterial taxa and functional genes present in the gut microbiome has matured to what will persist throughout most of adulthood. The adult microbiome is largely dominated two phyla, *Firmicutes* and *Bacteroidetes*. Later in life the gut microbiome appears to undergo some age-related changes characterized by a proliferation of opportunistic *Proteobacteria* at the expense of symbiont *Firmicutes* and *Bacteroidetes*; phyla that include species with recognized anti-inflammatory properties. It is fair to state that the precise nature and clinical significance of aging related changes in gut microbiota remain to be defined; given that many neurodegenerative diseases occur in the elderly, defining a "normal" older person's microbiota is of critical importance before we can ascribe any observed changes to disease.

Several studies have amply illustrated the impact of diet on the microbiome. First, studies comparing geographically diverse communities have ascribed differences in fecal microbial profiles to life-long dietary habits (7) and, second, differences in microbial fingerprints within communities or populations with similar demographics have been attributed to variable dietary habits (4,8). If sufficiently drastic, more short-to-medium term changes in diet (e.g. high protein, low fermentable oligo-, di-, or mono-saccharides and polyol [FODMAP's] diet, high vs low fiber or gluten-free) can also impact on microbiome composition (9). The adoption of other diets that exclude a single dietary component (e.g. lactose-, fructose- and sorbitol-free diets), or involve more extensive modifications (e.g. the Mediterranean or "paleo" diet), are likely to alter the composition of microbiota.

It is undoubted that interactions between diet, the indigenous microbiome and interventions that modulate the microbiome will continue to represent a major focus of future research.

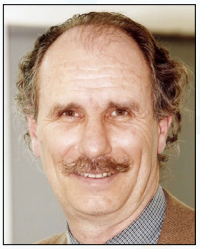
References:

1. O'Toole PW, Felmer B. Studying the microbiome: "Omics" made accessible. Semin Liver Dis 2016;36:1-6.
2. Quigley EMM. Gut microbiome as a clinical tool in gastrointestinal disease management: are we there yet? Nat Rev Gastroenterol Hepatol 2017;14:315-320.
3. Quigley EMM. Prebiotics and Probiotics in Digestive Health. Clin Gastroenterol Hepatol 2019;17:333-344.

1.2 The Gut Microbiome – an Introduction, continued.

4. Arumugam M, Raes J, Pelletier E, et al. Entero-types of the human gut microbiome. Nature 2011;473:174-80.
5. Collado MC, Rautava S, Aakko J, Isolauri E, Salminen S. Human gut colonization may be initiated in utero by distinct microbial communities in the placenta and amniotic fluid. Sci Rep. 2016;6:23129.
6. Perez-Munoz ME, Arrieta MC, Ramer-Tait AE, Walter J. 2017. A critical assessment of the "sterile womb" and "in utero colonization" hypotheses: implications for research on the pioneer infant microbiome. Microbiome 5:48.
7. Yatsunenko T, Rey FE, Manary MJ, et al. Human gut microbiome viewed across age and geography. Nature 2012;486:222-7.
8. Claesson MJ, Jeffery IB, Conde S, et al. Gut microbiota composition correlates with diet and health in the elderly. Nature 2012;488:178-184.
9. Clarke SF, Murphy EF, O'Sullivan O, et al. Exercise and associated dietary extremes impact on gut microbial diversity. Gut 2014;63: 1913-1920.

2.1 Functions of the Gut Microbiota



Francisco Guarner, MD, PhD
Digestive System Research Unit
Hospital Vall d'Hebron
Barcelona, Spain

Symbiosis in the Gastrointestinal Tract

Microbial colonizers of the gut are not casual bystanders, or potential invaders when immunity fails to keep them away. The normal interaction between gut microbes and their host is a symbiotic relationship, defined as mutually beneficial for both partners. The host provides a nutrient-rich habitat, and intestinal microbes confer benefits on the host's health. Evidence accumulated over the past decades indicates that gut microbial communities constitute an important functional part of animals. This was mainly proven by experiments using gnotobiotic rodents and birds.

Comparison of animals bred under germ-free conditions with their conventionally raised counterparts (colonized by conventional microbiota) has revealed a series of anatomic characteristics and physiological functions that are associated with the presence of the microbiota. Germ-free animals have extraordinary nutritional requirements in order to sustain body weight, and are highly susceptible to infections (Figure 1). Organ weights (heart, lung, and liver), cardiac output, intestinal wall thickness, gastrointestinal motility, serum gamma-globulin levels, lymph nodes, among other characteristics, are all reduced or atrophic in germ-free animals. Germ free mice display locomotor hyperactivity and reduced anxiety when compared with mice with a normal gut microbiota. Reconstitution of a germ-free animal with conventional microbiota by a fecal transplant restores most of these deficiencies, suggesting that gut microbes provide important and specific tasks to the host's homeostasis. Evidence obtained through such animal models suggests that the main functions of the microbiota are ascribed into three categories, i.e. metabolic, protective and trophic functions.

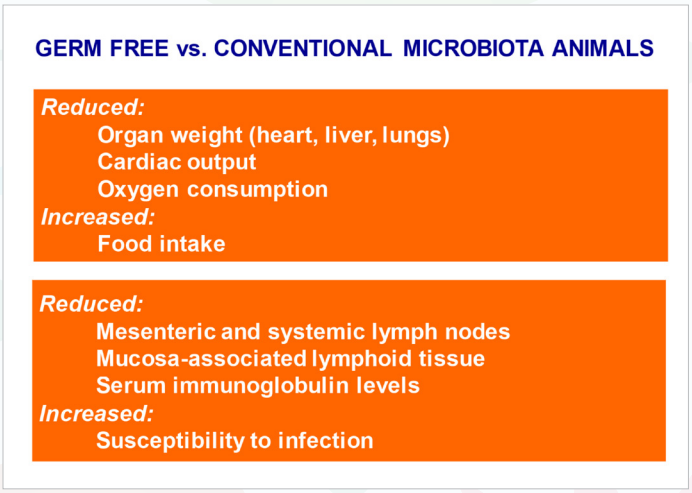


Figure 1: The impact of the microbiota on host anatomy and physiology is revealed in animals bred under germ-free conditions. When compared to conventionally colonized animals, germ-free animals have increased nutritional requirements in order to sustain body weight, are highly susceptible to infections and show structural and functional deficiencies. Reconstitution of germ-free animals with a microbiota restores most of these deficiencies, suggesting that gut bacteria provide important and specific tasks to the host's homeostasis.

Metabolic functions

The enteric microbiota has a collective metabolic activity equal to a virtual organ within the gastrointestinal lumen. Gene diversity among the microbial community provides a variety of enzymes and biochemical pathways that are distinct from the host's own constitutive resources.

For mammalians, the genes encoding enzymes for biosynthesis of many required organic compounds were lost early in evolution. Bacterial or fungal symbionts have, through evolution, adapted to provide the required organic compounds (essential amino acids and vitamins) and the ability to obtain energy from different sources. The guts of ruminants are well-studied examples of a host-microbe metabolic partnership. Symbiont communities carry out the task of breaking down complex polysaccharides of ingested plants, and provide nutrients and energy for both

2.1 Functions of the Gut Microbiota, continued.

microbiota and host. The amino acid supply of ruminants eating poorly digestible low protein diets largely depends on the microbial activities in their fore-stomach.

In the human being, the distal intestine represents an anaerobic bioreactor programmed with an enormous population of microbes. Due to the slow transit time of colonic contents, resident microorganisms have ample opportunity to degrade available substrates, which consist of non-digestible dietary residue and endogenous secretions. Colonic microbial communities provide genetic and metabolic attributes to harvest otherwise inaccessible nutrients.

Carbohydrates are fermented in the colon to short chain fatty acids, mainly, acetate, propionate and butyrate, and a number of other metabolites such as lactate, pyruvate, ethanol, succinate as well as the gases H_2, CO_2, CH_4 and H_2S. Short chain fatty acids acidify the luminal pH, which prevents the growth of pathogens, and favor the absorption of ions (Ca, Mg, Fe) in the cecum. They also influence intestinal motility and contribute towards energy requirements of the host. Acetate is metabolized in human muscle, kidney, heart and brain. Butyrate is largely metabolized by the colonic epithelium where it serves as the major energy substrate as well as a regulator of cell growth and differentiation.

The human proximal colon is a saccharolytic environment with the majority of the carbohydrate entering the colon being fermented in this region. In the distal colon, carbohydrate availability decreases, and proteins derived from desquamated epithelium become an increasingly important energy source for bacteria (Figure 2). Consequently, excessive fermentation of proteins in the distal colon has been linked with disease states such as colon cancer and chronic ulcerative colitis, which generally affect the distal region of the large intestine. Thus, it is recognized as favorable to shift the gut fermentation towards saccharolytic activity by increasing the proportion on non-digestible carbohydrates in the diet.

Protective functions

An important function of the gut microbiota was classically described as the 'barrier effect', which prevents invasion by pathogens. Resident bacteria represent a resistance factor to colonization by exogenous microbes or opportunistic bacteria that are present in the gut, but their growth is restricted. The equilibrium between species of resident bacteria provides stability in the microbial population, but antibiotics can disrupt the balance (for instance, overgrowth of toxigenic *Clostridioides difficile*).

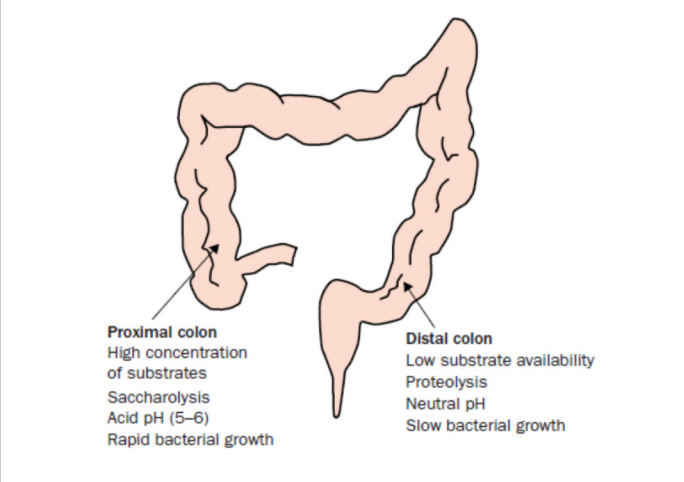


Figure 2: The human proximal colon is a saccharolytic environment. Fermentation of undigested carbohydrates is intense with high production of short-chain fatty acids, and rapid bacterial growth. By contrast, carbohydrate availability decreases in the distal colon and putrefactive processes of proteins are the main energy source for bacteria.

Several mechanisms are implicated in the barrier effect. Bacteria compete for attachment sites in the brush border of intestinal epithelial cells. Adherent non-pathogenic bacteria can prevent attachment and subsequent entry of pathogenic entero-invasive bacteria into the epithelium. Furthermore, bacteria compete for nutrient availability in ecological niches and maintain their collective habitat by regulating and consuming all resources. Elegant studies using mice mono-associated with *Bacteroides thetaiotaomicron* showed that the host provides a nutrient that the bacterium needs, and the bacterium actively indicates how much it needs to the host. This symbiotic relationship prevents unwanted overproduction of the nutrient, which would favor the intrusion of microbial competitors with potential pathogenicity for the host. Finally, bacteria can inhibit the growth of their competitors by producing antimi-

2.1 Functions of the Gut Microbiota, continued.

crobial substances called bacteriocins. The ability to synthesize bacteriocins is widely distributed among microbial collectivities of the gastrointestinal tract.

Trophic functions

These functions include the control of epithelial cell proliferation and differentiation, modulation of certain neuro-endocrine pathways, and the homeostatic regulation of the immune system. Epithelial cell differentiation is influenced by interactions with micro-organisms. Transcriptomic studies of intestinal mucosal biopsies reveal expression of a variety of genes in animals mono-associated with specific bacteria, and in humans fed with probiotic lactobacilli strains. Microbe interactions with epithelial cells produce distant effects. For instance, the microbiota suppresses intestinal epithelial cell expression of a circulating lipoprotein-lipase inhibitor, fasting-induced adipose factor (Fiaf), thereby, promoting the storage of triglycerides in adipocytes.

The ability of the gut microbiota to communicate with the brain and thus influence behavior is an unexpected exciting concept. Reports suggest that colonization by the enteric microbiota impacts mammalian brain development and subsequent adult behavior. In mice, the presence or absence of conventional enteric microbiota influences behavior, and is accompanied by neurochemical changes in the brain. Germ-free mice have increased locomotor activity and reduced anxiety, and this behavioral phenotype is associated with altered expression of critical genes in brain regions implicated in motor control and anxiety-like behavior. When germ-free mice are reconstituted with a microbiota early in life, they display similar brain characteristics as conventional mice. Thus, the enteric microbiota can affect normal brain development.

Gut microbes also play an essential role in the development of a healthy immune system. Animals bred in a germ-free environment show low densities of lymphoid cells in the gut mucosa and low levels of serum immunoglobulins. Exposure to commensal microbes rapidly expands the number of mucosal lymphocytes and increases the size of germinal centers in lymphoid follicles. Immunoglobulin producing cells appear in the lamina propria, and there is a significant increase in serum immunoglobulin quantities.

Most interestingly, commensals play a major role in the induction of regulatory T cells in gut lymphoid follicles. Control pathways mediated by regulatory T cells are essential homeostatic mechanisms by which the host can tolerate the massive burden of innocuous antigens within the gut or on other body surfaces without resulting in inflammation.

Studies in germ-free animals have clearly documented the key role of the microbiota in ensuring an optimal structural and functional development of the immune system. For instance, germ-free mice are immuno-deficient and highly susceptible to pathogen-mediated or opportunistic infections. In addition, they fail to develop normal adaptation to dietary antigens like ovo-albumin, and oral tolerance mechanisms are depressed or abrogated. These abnormalities can be corrected by reconstitution of a conventional microbiota, but this procedure is only effective in neonates and not in older mice. Massive interactions between gut microbial communities and the mucosal immune compartments early in life seem to be critical for a proper instruction of the immune system. Later in life, multiple and diverse interactions between microbes, epithelium and gut lymphoid tissues are constantly reshaping local and systemic immunity. Homeostasis of the host with the external environment seems to be highly influenced by the dynamic balance between microbial communities and the immune system.

The Human Gut Metagenome

Next generation DNA sequencing technology made it feasible to analyze the metagenome of the human gut, i.e. the total genetic content of the combined genomes of the microbial community, including bacterial and non-bacterial members (viruses, yeasts and protists). Up to 10 million non-redundant microbial genes have been identified. Each individual carries an average of 600,000 non-redundant microbial genes in the gastrointestinal tract, and around 300,000 genes are common in the sense that are present in about 50% of individuals (Table).

The catalog of microbial genes encodes groups of proteins engaged in up to 40,000 biological functions related with life in the intestinal habitat. The minimal functional metagenome found in all individuals includes 6,000 of those functions. Some functions are common to free-living

2.1 Functions of the Gut Microbiota, continued.

Microbial Genes in the Human Gut	Number of genes
Catalog of non-redundant microbial genes	9,879,896
Common genes (present in at least 50% of individuals)	294,110
Median gene set per human individual	590,384
Median set of common genes per individual	204,056
Microbial Functions in the Human Gut	**Number of functions**
Annotated gene functions according to KEGG and eggNOG databases	43,469
Median set of functions per individual	6,313

TABLE: The Human Gut Metagenome

bacteria, like the main metabolic pathways (e.g. amino-acid synthesis, RNA and DNA polymerases, ATP synthase, general secretory apparatus). Some other gene clusters encode functions that may be especially important for microbial life within the gut, such as those involved in adhesion to host proteins (collagen, fibrinogen, fibronectin) or harvesting sugars from the glycolipids secreted by epithelial cells.

References

Gilbert JA, Blaser MJ, Caporaso JG, Jansson JK, Lynch S V., Knight R. Current understanding of the human microbiome. Nat Med. 2018;24(4):392–400.

Heijtz RD, Wang S, Anuar F, Qian Y, Bjorkholm B, Samuelsson A, et al. Normal gut microbiota modulates brain development and behavior. Proc Natl Acad Sci. 2011;108(7):3047–52.

Hooper LV, Macpherson AJ. Immune adaptations that maintain homeostasis with the intestinal microbiota. Nat Rev Immunol. 2010 Mar;10(3):159-69.

Kelly JR, Keane VO, Cryan JF, Clarke G, Dinan TG. Mood and Microbes: Gut to Brain Communication in Depression. Gastroenterol Clin North Am. 2019;48(3):389–405.

Li J, Wang J, Jia H, Cai X, Zhong H, Feng Q, et al. An integrated catalog of reference genes in the human gut microbiome. Nat Biotechnol. 2014;32(8):834–41.

van Baarlen P, Troost F, van der Meer C, Hooiveld G, Boekschoten M, Brummer RJM, et al. Human mucosal in vivo transcriptome responses to three lactobacilli indicate how probiotics may modulate human cellular pathways. Proc Natl Acad Sci. 2010;108(Supplement_1):4562–9.

Wostmann BS. The germfree animal in nutritional studies. Annu Rev Nutr. 1981;1:257-79.

2.2 Techniques to Characterize Gut Microbiota

Paúl Cárdenas, MD, PhD

Associate Professor
Institute of Microbiology
Universidad San Francisco de Quito
Quito, Ecuador

Introduction

Most of the microorganisms present in a natural environment, including the gut microbiota in humans, live in mixed populations. Standard laboratory culture techniques do not always result in successful bacterial identification and around of 80% of bacteria cannot be cultured by standard methods. Consequently, the most powerful approach to study the microbial diversity is with the implementation of molecular culture-independent techniques. These techniques have exhibited robust results that not only show correlation between the gut microbiota and disease but have rationalized causality. The application of these techniques has allowed to identify distinctive gut microbiota patterns among healthy and diseased individuals. However, in the recent years, the studies have expanded from the ecological characterization of the gut microbiota (the differences of bacterial and/or fungal diversity and/or abundance between samples) to the functional characterization of the microbiota.

Sampling methods

The archetypal gut microbiota sample is feces, however along the gastrointestinal tract are present different ecological niches. Fecal samples are not the most reliable samples to characterize the lower intestine resident microbial communities but are the easiest samples to access for clinical research. Systematic bias can be introduced when samples cannot be frozen at -80 Celsius in at least 72 hours after collection (immediate freezing is recommended). Still preservatives like ethanol 95% and RNAlater can be used to maintain samples stability at room temperature for up to 7 days without important changes in the microbiota composition. Additionally, the within structure of fecal matter has different environmental niches that harbor different bacteria (i.e: anaerobes inside and aerobes outside), for that reason it is recommended to homogenize the entire sample prior DNA extraction. The fecal samples are subject specific, for that reason the better way to make them a good gut microbiota representative is by designing better controlled studies, with larger number of individuals and on a prospective manner.

The stomach and the small intestine usually are underrepresented on the gut microbiota studies mainly because they are difficult to reach, however the upper gut microbiota is very different compared to the lower intestine or the fecal samples. In order to sample the stomach or the small intestine, it is necessary to use invasive sampling methods as esophagoduodenogastroscopy, colonoscopy, luminal brushing or even surgical intestinal resection. Other methods have been carried on, like direct small intestine microbiota analysis in patients with ileostomies still with limited number of patients and very likely skin contamination.

Nowadays new non-invasive strategies have been developed like robotic swallowable capsules that contain pH and temperature sensors, and independent batteries. These capsules can take microbiota samples from specific points and as the technology develops, the costs are getting more accessible. However, there are still some issues that have not been resolved with these devices like contamination (from other than the desired sites) or sample preservation.

Sampling is a crucial step on microbiota research, for that reason it is important not only to plan carefully the sampling methods, but to take account strict inclusion and exclusion criteria to avoid skewed results. For example, dysbiosis can be caused by current infectious processes, recent use of antibiotics or other medicines, diet variations, comorbidities, etc.

From bacterial/fungi gene marker identification to shotgun-metagenomics

The conventional and still most used technique to characterize the gut microbiota is the amplification of a universal gene marker as 16S rRNA gene for bacteria and ITS for fungi. The limitations of this approach are founded in bias

2.2 Techniques to Characterize Gut Microbiota, continued.

introduced by PCR (polymerase chain reaction), the region of the gene sequenced (usually Illumina technologies provide a high sequencing coverage but short fragment size sequencing), and existing information on databases (there are issues in taxonomic assignments depending of the gene and/or gene fragments used). The majority of the molecular tools used for the characterization of the bacterial microbiome are based on the genomic evolutionary relationships (mainly in the similarities of housekeeping genes) between the bacterial genomes. Comparison of the 16S rRNA gene is the most popular technique to classify bacteria phylogenetically due to its highly conserved sequence and the ease with which evolutionary relationships can be identified. Besides, the gene encoding the bacterial 16S rRNA variable subunits allow universal PCR amplification and sequencing of bacteria from clinical samples. Using this approach bacteria can be assigned to a specific genus and, in some cases, to a specific specie by comparing the obtained sequence to those of already characterized species in the GeneBank and Ribosomal DNA databases. Taxonomic assignation still can be a problem when encountering new bacteria or fungi, or in some cases the sequence homogeneity on these genes does not allow to discriminate differences at lower taxonomic level as in many bacteria the core genome (including housekeeping genes) is a small proportion on the pangenome.

The use of gene markers allows to determine ecological features in the gut microbiota as diversity and abundance of specific bacteria or fungi. This means that this approach allows to describe what are the main microorganism present and to compare patterns between individuals or groups. The most common ecological parameters measured are the alfa diversity (number of species and uniformity of species calculated within each individual), beta diversity (differences of presence/absence, abundance or phylogenetic distances compared between people whole microbiota) and relative abundance (differences in proportions of bacteria or fungi types compared between groups).

Shotgun metagenomics is more frequently used to determine the gut microbiota composition avoiding the possible bias introduced by PCR amplification of a gene marker. This approach consists in directly sequence all the DNA (and sometimes RNA) present in a sample without a prior gene marker selection. It also broadens the spectrum of microorganisms studied in the same sample as it is not necessary to run different reactions for each taxonomic kingdom (one for bacteria and another for fungi) but both can be analyzed at the same time. Additionally, the 'dark matter' of microbiota (host viruses, phages and previously not described DNA fragments) can also be analyzed in the same experiment. It provides a more compressive analysis of the gut microbiota ecological features. Also, the use of long-read sequencing technologies has boosted, for that reason identification of longer fragments allow better taxonomic assignation. As consequence, the use of operational taxonomic units (OTUs) or amplicon sequence variants (ASVs) will drop in short time and direct assignation of species used. Supplementary to ecological features description, shotgun metagenomics allow to report the metabolic and functional genes from bacteria and fungi present in the microbial community (Figure 1).

Problems with shotgun metagenomics are originated in the deepness of sequencing necessary to identify the less common microorganism present in the community. It is important to take account that lower deepness could

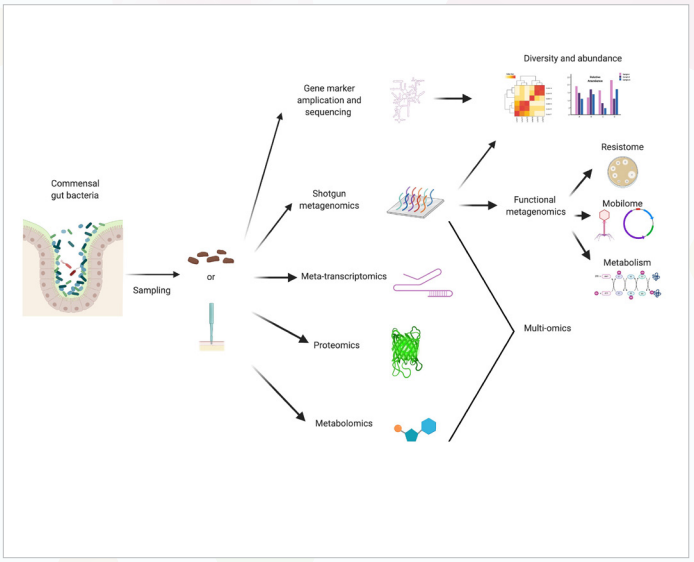


Figure 1. Summary of techniques used to characterize the gut microbiota. Created by Biorender.com

2.2 Techniques to Characterize Gut Microbiota, continued.

provide less information about the less common microorganisms present in the gut microbiota, than the gene marker approach. Another issue with shotgun metagenomics is that depending on the sampling method, lots of sequences produced can come from the human host instead of the microbial community. This is more evident in samples that contain human tissue as biopsies or luminal brushing. Even though gut microbiota is the most studied ecological niche compared to environmental samples, still databases show incomplete or unknown information. As a result, more information is produced but most of it cannot be used.

Functional metagenomics

Apart from new sequencing technologies, bioinformatics tools made possible to construct a better understanding on the functional aspects of the gut microbiota. Tools as pycrust2 allow to infer the metabolic and pathogenic genes on a microbial community based on the ecological features (obtained from marker genes microbiota studies). Even it is not a direct characterization of function, this tool helps to understand the metabolic changes that probably occur in dysbiotic events.

Shotgun metagenomics directly allows the functional characterization of microbial communities, such as metabolic and virulence genes. If we consider the microbial communities as a whole, understanding the functional characteristics of the microbiota is more important than the ecological aspects. Besides, to complement with sequencing, bioinformatic pipelines have been created to characterize specific functional features as antibiotic resistance genes (appointed as the resistome) or mobile genetic elements (named the mobilome). These approaches use particular databases that are mapped to the sequences obtained and allow to identify and quantify the genes of interest.

Resistome dynamics are important characteristics driven by the `one health´ hypothesis, and the evidence of antibiotic resistance genes transfer between humans, animals and environment. Regarding the mobilome a combination of long and short reads sequencing is necessary in order to determine the plasmid/transposon/integron structure. Phages are important actors in bacterial horizontal gene transfer; however, it is still unknown if they play an important role on antibiotic resistance genes transfer as they do in virulence genes transmission (Figure 1).

Multi-omics

Multi-omics or integrative omics (genomics-transcriptomics-proteomics-metabolomics) is becoming an important and accessible tool to fully characterize the microbiota functionality related with disease and additionally the intra-individual changes that happens over time independently of any distress (disease, antibiotics, diet changes, etc.). Each experiment is made individually (each experiment has its own pipeline and sometimes multiple samples have to be taken from the same individual), and every result is analyzed jointly in order to create networks that recreate the microbiota physiology. It is also interesting that a multi-omics approach leads to understand not only the microbial community intrinsically but also its relationship with the host (Figure 1).

Principal challenges with this approach are related to integrate different types of data produced by a wide number of experiments in conjunct outcomes. Marginal correlation analysis, regression-based methods and Gaussian or Bayesian models are the statistical approaches used to resolve relations between genes, transcripts, proteins, and metabolites inferred from multiple statistically independent observations. As expected in a new field, these network statistical methods are improved and questioned regularly until finding a method that fits with the physiology encountered in the gut microbiota.

Further reading

Bengtsson-Palme J, *et al*. Using metagenomics to investigate human and environmental resistomes. J Antimicrob Chemother. 2017;72(10):2690-2703. doi:10.1093/jac/dkx199

Jiang D, *et al.* (2019) Microbiome Multi-Omics Network Analysis: Statistical Considerations, Limitations, and Opportunities. Front. Genet. 10:995. doi: 10.3389/fgene.2019.00995

Tang Q, *et al*. (2020) Current Sampling Methods for Gut Microbiota: A Call for More Precise Devices. Front. Cell. Infect. Microbiol. 10:151. doi: 10.3389/fcimb.2020.00151

2.2 Techniques to Characterize Gut Microbiota, continued.

V. R. Carr, *et al*. Probing the Mobilome: Discoveries in the Dynamic Microbiome. Trends Microbiol., Aug. 2020, doi: 10.1016/j.tim.2020.05.003.

Wang *et al*. Application of metagenomics in the human gut microbiome. World J Gastroenterol. 2015;21(3):803-814. doi:10.3748/wjg.v21.i3.803

2.3 Composition and Structure of Human Gut Microbiota Along the Gastrointestinal Tract

Gerald Holtmann, MD, PhD, MBA, FRACP, FRCP, FAHMS

Professor of Medicine
Director of Gastroenterology and Hepatology
Princess Alexandra Hospital
Director of Clinical Innovation
University of Queensland
Brisbane, Australia

Ayesha Shah, MBBS, FRACP

Senior Lecturer University of Queensland
and Senior Medical Officer
Department of Gastroenterology and Hepatology
Princess Alexandra Hospital
Brisbane, Australia

Mark Morrison, PhD

Professor and Chair of Microbial Biology and Metagenomics
Diamantina Institute
University of Queensland
Science Lead in Gastrointestinal Function
Princess Alexandra Hospital
Brisbane, Australia

Introduction

The entire human gastrointestinal (GI) tract is colonised by dynamic and complex populations of microbes providing a range of goods and services that affect host gut homeostasis, nutrition and metabolism, and systemic function. The gut microbiome thereby influences the host's predisposition or susceptibility for specific diseases. The gut microbiome is shaped by many factors and most dynamic in early life. It can be affected by mode of delivery, choice between breastfeeding or milk replacement formula, duration of the preweaning period, antibiotic and medications, post-weaning dietary pattern, exposure to parents, siblings, and companion animals, and a myriad of other lifestyle and environmental factors overlaid on their genetic background. Although these communities show some degree of "stabilization" post-infancy and as their immune system matures, the relative abundance of microbes that colonise the various segments of the gastrointestinal tract can be further shaped throughout life by environmental and lifestyle choices, and in particular, by diet. In summation, the gut microbiome is the "x-factor" in the genotype x environment x lifestyle interactions that affect host phenotype. While much information about its features has now been acquired, the microbiome still remains a black box of undefined functional attributes which remain cryptic in terms of their relevance and impacts on our health and well-being.

The journey from mucosal to luminal and faecal microbiome

For much of the last two decades, the term "gut microbiome" has become to refer almost exclusively to those microbes recovered from stool samples. The relatively easy access to stool samples and its invariably large amounts of microbial biomass makes it an "attractive" resource for study. However, it is now widely recognized that the microbes that specialise in colonising the mucosa at different segments of the gastrointestinal tract are not only different, but perhaps even more relevant in driving host responses relevant to health and disease. Indeed, many alterations of the stool microbiome might reflect underlying functional abnormalities rather than the cause of these alterations. Thus, alterations in the stool microbiome in patients with constipation might simply reflect those microbes more capable of adapting to the physicochemical conditions associated with slow digesta transit rather than being the cause of constipation. Similarly, patients with diarrhea impose a strong selective pressure towards those microbes capable of growing most rapidly on the dilute nutrients available. Under both conditions, those microbes capable of adhesion to the mucosa might be considered to reside within a more stable microenvironment and as such, remain largely unchanged in terms of diversity and density. Much more needs to be learned about the mucosa-associated microbiota along the entire gastrointestinal tract and how any region-specific variations in these communities affects our health and well-being.

2.3 Composition and Structure of Human Gut Microbiota Along the Gastrointestinal Tract, continued.

Factors that determine the composition of the microbiome along the gastrointestinal tract

Microbial colonisation of various segments of the gastrointestinal tract has long been known to be influenced by the physiochemical conditions that typify these various segments. For example, the luminal contacts and mucosa of the stomach is characterised by a very low gastric pH, while in the duodenum pH is rapidly increased due to pancreatic bicarbonate secretion into the 2nd part of the lumen. While the growth and persistence of many types of gut microbes are sensitive to acidification, others have evolved to adapt and withstand this challenge. Two classic examples are lactic acid bacteria (e.g. Lactobacilli) and *Helicobacter pylori*. The survival of *H. pylori* in the stomach is attributable to its urease activity, which is an enzyme that converts urea to ammonia, and creates a more alkaline "cloud" at its site of colonization on the gastric mucosa. In addition, the pH changes occur across the mucus layer covering surfaces of human gastric mucosa[1]. While the luminal pH might be very low in the stomach, the mucous layer provides cover for acid sensitive microbes due to this pH gradient with low pH on the luminal side and higher pH near the mucosa. Indeed, the microbes present in the intestinal lumen differ significantly from the microbiota attached and embedded in this mucus layer as well as the microbiota present in the immediate proximity of the epithelium[2-4]. Thus, specific microbes that are pH sensitive may survive below a protective mucus layer while – when exposed directly to luminal content – growth would be substantially impaired. Even in in the second part of the duodenum - where the acid secreted into the stomach that is subsequently emptied into the duodenum is neutralised by pancreatic bicarbonate – the composition of microbes colonising the surface of the small intestine or microbes that colonise the mucosa below the mucus layer might be different. Indeed, a study that compared microbial profiles on the surface (captured by a sterile brush) revealed subtle differences as compared to profiles of mucosal biopsies utilising aseptic sampling techniques or traditional biopsy techniques (Figure 1)[4]. Thus, in addition to longitudinal heterogeneity displayed by the intestinal microbiota, there is also a great deal of latitudinal variation in the microbiota composition.

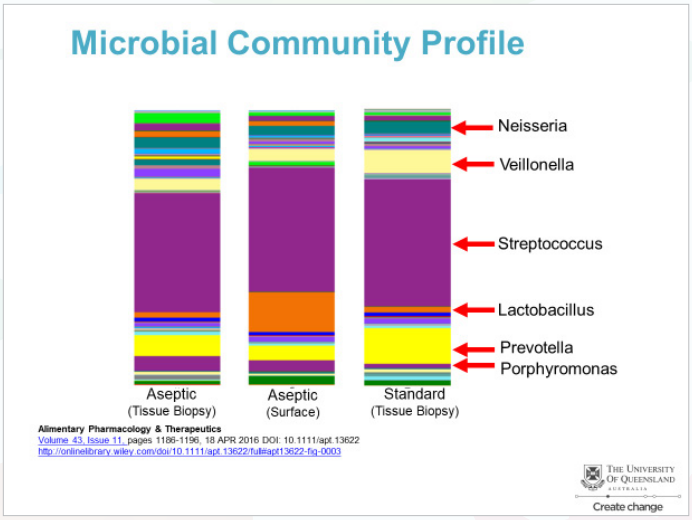


Figure 1: relative abundance of various microbial communities found in a given subject in the 2nd part of the duodenum when sample are obtained by superficial brushing, aseptic biopsies or biopsies obtained with a normal biopsy device potentially cross contaminated (with permission from Shanahan ER, Zhong L, Talley NJ, Morrison M, Holtmann G. Characterisation of the gastrointestinal mucosa-associated microbiota: a novel technique to prevent cross-contamination during endoscopic procedures. Aliment Pharmacol Ther. 2016 Jun;43(11):1186-96)

In addition to pH and endogenous secretions (e.g. bile, lipases, other pancreatic digestive enzymes, and endocrine peptides) a variety of other factors will influence microbial growth along the gastrointestinal transit. The most obvious of these is diet, which is most often described in terms of the relative amounts of fibre, carbohydrate, fat, and protein. Indeed, the influences of diet on the stool microbiome are perhaps among the most intensively studied and extensively reported aspect of "gut microbiome research". When food is ingested, nutrients are released and absorbed as the chyme is exposed to digestive enzymes. The rate of absorption is different for various nutrients. If e.g. fat is infused into the proximal duodenum only 20% reaches the distal duodenum, whereas 50% of protein and 60 % carbohydrates remain in the lumen. In parallel with nutrient absorption, the luminal enzyme activities decrease due to autodigestion contributing to distinct microenvironments in various segments of the gastrointestinal tract

2.3 Composition and Structure of Human Gut Microbiota Along the Gastrointestinal Tract, continued.

that directly relate to the ability of specific microbes to colonise the gastrointestinal tract[5]. Furthermore, in the small intestine the concentration of digestive enzymes and bile is high, and this may adversely affect growth of microorganisms while bile concentrations and enzyme activities decrease during transit from the duodenum to the colon. In addition, transit in the small intestine is relatively rapid. This limits the bacterial density in the small intestine and explain why motility disorders of the small intestine can be associated with conditions such as small intestinal bacterial overgrowth (e.g. increased bacterial load in the small intestine). All these factors explain differences in the density of bacteria in the small intestine and colon[6].

Bacterial colonisation of different segment of the Gut

The gastrointestinal tract starts with the oral cavity and the oral cavity harbours microbes that appear to influence microbial communities in the GI tract and subsequently influences human health [7]. The density of bacteria present in the luminal contents of the mammalian gut display a density gradient that ranges from 10^1 to 10^3 bacteria colony forming units per ml (cfu/ml) in the stomach and duodenum, to 10^4 to 10^7 bacteria cfu/ml in the jejunum and ileum, to 10^{11} to 10^{12} cfu/ml in the colon[8] (Figure 2). In the stomach, the microbiome is predominantly comprised of Gram-positive and microaerophilic bacteria such as Streptococci, Staphylococci, Lactobacilli, and various fungi. Many of these taxa are also represented within the communities of the oropharynx[9]. The small intestine constitutes a zone of transition between the sparsely populated, acidic stomach and the dense and diverse variety of microbes resident within the colon. Under normal conditions the microbiota of the proximal small bowel are more similar to those resident in the stomach noted above, but expanded to include *Veillonellaceae* and *Actinomycetales*. In the distal ileum, the distribution between bacterial taxa deemed Gram-negative and Gram-positive becomes more balanced, and anaerobic bacteria such as *Bacteroides, Bifidobacterium, Fusobacterium,* and *Clostridium* spp. are found in substantial concentrations[9-11]. Thus, in the terminal ileum distal to the ileocecal sphincter bacterial concentrations increase sharply. Within the colon, the bacterial concentration is 10^{11} to 10^{12} cfu/ml, and are predominantly fastidious anaerobes affiliated with four key Phlya: Firmicutes, Bacteroidetes, Actinobacteria and Proteobacteria[9, 12-15].

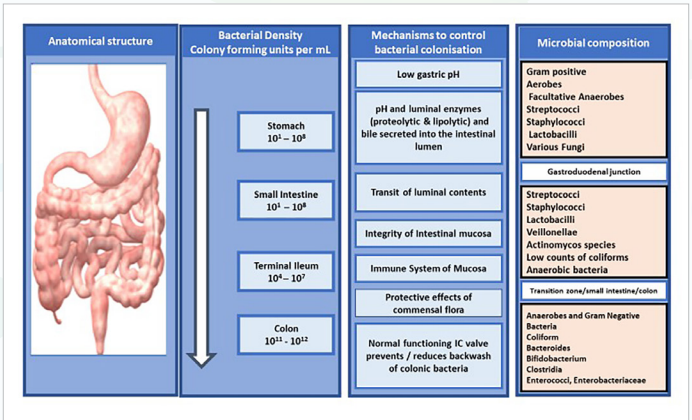


Figure 2: Composition of the Gut Microbiota in the different parts of gastrointestinal tract and the factors that influence the density and composition of bacterial colonisation.

In conclusion there are substantial differences in the composition and density of microbes colonising different segments of the human gastrointestinal tract. It is evident that physicochemical factors such as pH, bile, enzyme and nutrient concentrations, as well as host factors (e.g. mucosal immune function) influence the microbial colonisation. The microbes that reside in these different sites are likely to play a critical role to shape immune responses and impart important impacts on metabolism and nutrtion via the good and services they provide. The complexity of these host-microbe interactions are now amenable to description but resilient to understanding and management with predictable clinical outcomes. While an incredible amount of insight and methodological/analytical expertise has been gained from the study of the stool microbiota, we are just at the beginning of realizing how the complexity of the gastrointestinal microbiota can be monitored and manipulated to deliver health outcomes. Hopefully, there will continue to be an "expansion" of the research of the gastrointestinal microbiota, both in a longitudinal (i.e. proximal to distal) and latitudinal (luminal to mucosal) perspective.

2.3 Composition and Structure of Human Gut Microbiota Along the Gastrointestinal Tract, continued.

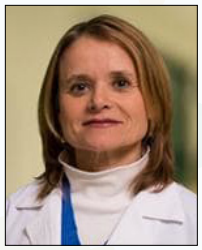
References:

1. Bahari HM, Ross IN, Turnberg LA. Demonstration of a pH gradient across the mucus layer on the surface of human gastric mucosa in vitro. Gut 1982;23:513-516.
2. Swidsinski A, Loening-Baucke V, Lochs H, et al. Spatial organization of bacterial flora in normal and inflamed intestine: a fluorescence in situ hybridization study in mice. World J Gastroenterol 2005;11:1131-40.
3. Sekirov I, Russell SL, Antunes LCM, et al. Gut Microbiota in Health and Disease. Physiological Reviews 2010;90:859-904.
4. Shanahan ER, Zhong L, Talley NJ, et al. Characterisation of the gastrointestinal mucosa-associated microbiota: a novel technique to prevent cross-contamination during endoscopic procedures. Aliment Pharmacol Ther 2016;43:1186-96.
5. Holtmann G, Kelly DG, Sternby B, et al. Survival of human pancreatic enzymes during small bowel transit: effect of nutrients, bile acids, and enzymes. Am J Physiol 1997;273:G553-8.
6. Guarner F, Malagelada JR. Gut flora in health and disease. Lancet 2003;361:512-9.
7. Gao L, Xu T, Huang G, et al. Oral microbiomes: more and more importance in oral cavity and whole body. Protein Cell 2018;9:488-500.
8. O'Hara AM, Shanahan F. The gut flora as a forgotten organ. EMBO reports 2006;7:688-693.
9. Gorbach SL, Plaut AG, Nahas L, et al. Studies of intestinal microflora. II. Microorganisms of the small intestine and their relations to oral and fecal flora. Gastroenterology 1967;53:856-67.
10. Drasar BS, Shiner M, McLeod GM. Studies on the intestinal flora. I. The bacterial flora of the gastrointestinal tract in healthy and achlorhydric persons. Gastroenterology 1969;56:71-9.
11. Drasar BS, Shiner M. Studies on the intestinal flora. II. Bacterial flora of the small intestine in patients with gastrointestinal disorders. Gut 1969;10:812-9.
12. Finegold SM, Attebery HR, Sutter VL. Effect of diet on human fecal flora: comparison of Japanese and American diets. Am J Clin Nutr 1974;27:1456-69.
13. Simon GL, Gorbach SL. Intestinal flora in health and disease. Gastroenterology 1984;86:174-93.
14. Hill MJ, Drasar BS. The normal colonic bacterial flora. Gut 1975;16:318-23.
15. Simon GL, Gorbach SL. The human intestinal microflora. Dig Dis Sci 1986;31:147S-162S.

2.4 Acquisition of the Human Gut Microbiome

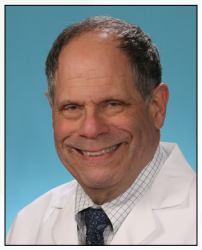
Maria Antony, MD

Department of Medicine
UConn Health
Farmington, Connecticut, USA



Barbara B. Warner, MD

Department of Pediatrics
Washington University
School of Medicine in St. Louis
St. Louis, Missouri, USA



Phillip I. Tarr, MD

Department of Pediatrics
Washington University School of Medicine

Department of Molecular Microbiology
Washington University School of Medicine
St. Louis, Missouri, USA

Yanjiao Zhou, MD

Department of Medicine
UConn Health
Farmington, Connecticut, USA

Introduction

The gut microbiome dynamically evolves throughout life. Early-life microbiome development plays major roles in future health, highlighting the importance of understanding the evolutionary processes of this community of microbes. This is a dynamic area of study **(Figure 1)**. Here, we review recent data on early-life microbiome acquisition patterns, critical factors shaping the microbiome trajectory, and discuss necrotizing enterocolitis (NEC) as a paradigmatic microbial dysbiosis-driven disorder.

Important roles of early-life colonizers in host health

Acquisition of the gut microbiome in early life lays the foundation for future health. Early life microbial colonizers include bacteria and viruses that interact with one another and the host. These organisms support organ homeostasis, immune tolerance and metabolic regulation. Internal and external insults can jeopardize the healthy development of gut microbial communities and lead to serious disease. This is well exemplified by NEC, a life-threatening microbiome dysbiosis-associated disorder that afflicts preterm infants in the first two months of life [1,2]. Data are also accumulating that later-in-life immune disorders, such as asthma [3] and type 1 diabetes [4] are at least partly associated with early-life gut microbiome dysbiosis.

Earliest-in-life microbial colonizers

High throughput sequencing technology that profiles both microbial populations and their genetic and inferred metabolic repertoires has altered our view of sterility in a healthy utero environment. A number of microbiome studies suggest the presence of microbial DNA in amniotic fluid and placenta in normal pregnancies for infants delivered at term [5 6–8]. The bacterial DNA identified infers a low diversity community, and resemble the vaginal, oral or intestinal microbiomes, indicating potential routes of microbial transference to the uterus. However, concerns about DNA contamination from various sources have been raised and slowed acceptance of the utero-origin of the microbiome [9–12]. Regardless, the search for an *in utero* microbiome continues with approaches focusing on identification of variable bacteria and live-dead differentiation methods [13]. Until we can resolve the issue of DNA contamination, the debate of the prenatal origin of the infant gut microbiome will continue.

In contrast, *in utero* microbial presence and preterm delivery are generally considered to be a more common association, and pathogenic finding [14]. Compared to term delivery, infants born preterm have higher bacterial prevalence [15,16], and harbor higher relative abundances of Proteobacte-

2.4 Acquisition of the Human Gut Microbiome, continued.

ria and vaginal microbiota in placenta and amniotic fluid [5,7,15,17,18]. Bacteria commonly found in amniotic fluid and placenta have been proposed to be transmitted from vaginal or oral sites, and are associated with intra-amniotic infection and preterm delivery [19,20].

Following amniotic sac rupture, the infant is next exposed to microbial inoculation from the mother's vagina or skin, depending on delivery mode and the interval between rupture and delivery. However, the impact of delivery mode on the microbiome is also subject to debate. Early work supports that microbial sequences in meconium microbiome at birth are indistinguishable from other body sites and the microbiome is more similar to those in maternal skin and vagina, respectively [21]. However, subsequent work reported no discernable effect of mode of delivery on meconium microbiome or gut microbial content at six weeks of age [22]. In addition, the duration of the reported impact of delivery mode on the gut microbiome varies from several weeks to 2 years [23 24,25 26]. Vaginal seeding, a procedure that transplants vaginal liquid to infants born by C-section is being explored to mimic microbial exposure of vaginal delivery, but its effects and risks are incompletely assessed [27].

In term infants, the earliest colonizers of the stool microbiome are facultative bacteria such as *Lactobacillus, Propionibacterium, Streptococcus, Staphylococcus, Enterobacter, Escherichia* [22,28], which are soon succeeded by obligate anaerobes, such as *Bifidobacterium, Enterobacteriaceae, Bacteroides,* and *Clostridium*, which persist [28,29]. However, obligate anaerobic bacteria, *Roseburia, Faecalibacterium* (major butyrate producers) [29], and *Akkermansia* (a well-known mucin degrader) [30], are absent during the neonatal period. Gut microbiome diversity increases from birth to 6 months of age [23].

Introduction of formula milk and table food around 5-6 months of age in a term infant alters the gut microbiome significantly, as demonstrated by marked increase of *Bacteroides*, a major bacterial genus in adults[31]. Meanwhile, the abundance of *Bifidobacterium* starts decreases more profoundly by 18 months [30]. The infant gut microbiome becomes stable and adult-like in configuration at about 2-3 years of age [31,32]. Notably, the gut microbiome has the metabolic capacity for plant-derived glycans metabolism prior to the introduction of solid foods [31].

Gestational age is a major factor that affects the microbiome in early life. Compared to term infants, preterm infants have distinct microbiome exposure such as long-term stays in a neonatal intensive care unit (NICU), prolonged antibiotic administration, parenteral nutrition and lack of physical contact [2]. The gastrointestinal tracts of preterm infants harbor significantly greater abundances of Proteobacteria than term infants at week 1 of age, and experience delayed in-population by anaerobic bacteria (*Bifidobacterium, Bacteroides*) and higher abundances of *Enterococcus* and other genera that contain potentially opportunistic pathogens [33–38]. However, an orchestrated shift of bacterial class from Bacilli to Gammaproteobacteria to Clostridia and Negativicutes is still evident in preterm infants in the first two months of age [39]. The microbiome diversity of preterm infants increases over 6 months of study period [37], but more gradually and with greater variation, compared to term infants [40]. Although delayed, the gut microbiome compositions and diversity in preterm infants largely resemble those in term infants at 21 months of age [40].

Eukaryotic viruses and bacterial phages are also important components of the gut microbiome. Recent studies reveal no virome in amniotic fluid [11]. Virus-like particles are not detected in meconium and early stool samples, but appear by one month of age (mostly prophages). These prophages are likely induced in early bacteria colonizers. Virus replication in human cells become more prominent by 4 months, demonstrating a stepwise virome colonization pattern[41]. Like the bacterial microbiome, the diversity of the eukaryotic virome expands from birth to 2 years, in contrast to the diversity of bacteriophage that predominate at birth and dissipate over time[42]. The virome development in preterm infants remains under-studied.

It is important to note that the patterned microbiome development is often punctuated by unpredictable and abrupt disruption, which cannot be explained by known host factors, except for use of selected antibiotics, especially in infants born preterm [43,44]. There is considerable inter-individual variability of the gut microbiome during

2.4 Acquisition of the Human Gut Microbiome, continued.

development [30], with stochastic elements contributing to this variation. [45].

Breast feeding and antibiotics

Microbiome composition and trajectory in early life are shaped by a complex array of maternal and infant conditions. Maternal diet, BMI, and health conditions influence the gut microbiome composition in the early days of life [46,47], but the impact is less influential than host (infant) factors, such as infant diet and antibiotic use [48,49].

Infant diet has profound and long-lasting impact on the gut microbiome development. Human milk contains rich nutrients, distinctive bioactive molecules, and live microbes, indicating that it is the optimal diet for the early development. One meta-analysis of papers consisting of 1,825 stools from 684 infants shows exclusive breast-feeding and non-exclusive breast-feeding as determinants in gut bacterial diversity, specific taxa, and microbial functional potential [50]. The microbiota age is younger in breastfed infants at 4 months of age, dominated by bacteria such as *Lactobacillus* and *Bifidobacterium*. Breastfed infants exhibit higher levels of oxidative phosphorylation and vitamin synthesis, whereas formula-fed infants are functionally enriched in bile acid biosynthesis, methanogenesis and short-chain fatty acid metabolism [28,51]. Interestingly, amino acid synthesis pathways in the gut microbiome of breastfed infants coincide with the changing composition of amino acid content in breast milk [51]. The probiotic-like microbial community maintains dominance at 12 months old in infants who continue to breastfeed, which is in contrast with formula-fed infants who accrue adult-like bacteria [28].These findings suggest that infant diet has a strong influence on microbial community structure and functionality. In addition, compared to the mode of delivery (the effects of which appear to be time-limited), specific feeding may persistently influence gut microbiome composition (i.e. *Bacteroides*) into adulthood [52].

Compared to formula feeding, donor milk and mother's own milk favorably alters the composition and diversity of the microbiome, as demonstrated by the greater diversity and significantly lower relative abundance of Enterobacteriales, and higher abundances of Clostridiales, Lactobacillales, *Bifidobacterium*, and Bacillales in preterm infants at 4-6 weeks of age [53–55]. Interestingly, the gut microbiome profiles of preterm infants fed by donor milk more closely resemble those of the mother's own milk than those of infants fed formula [55]. After adjusting for differences in gut maturity, an ordered succession of microbial phylotypes with the first 60 days of life occurs in breastfed infants, which appears to be disrupted in infants who consume formula alone [56].

Antibiotic administration disrupts the microbiome community and enriches antibiotic resistance genes during microbiome development in term infants. Broad-spectrum antibiotics administered at the beginning of life greatly affect the gut microbiome for at least the next 1 to 6 months, with decreased microbial diversity, delayed maturation, overgrowth of Proteobacteria, and attenuation of *Bifidobacterium* [25,57,58]. In many cases, single strains predominate after antibiotic treatment [30], and microbial diversity recovers over the first year of life [26]. Compared to feeding and the mode of delivery, antibiotics have smaller effects on microbial diversity [26].

Historically, nearly all preterm infants receive intensive and prolonged antibiotic treatment in the first month of life. Similar to term infants, antibiotic exposure decreases microbial diversity with the exception of gentamicin, and delays microbiome maturation [40,43]. Persistence of distinct antibiotic-driven patterns of microbiota and multi-drug resistance of *Enterobacteriaceae* are found in hospitalized preterm infants [40]. Intermediate-term follow-up shows that gut carriage of multi-drug resistant bacteria diminishes in preterm infants by 2 years of age [59], but multi-drug resistant Enterobacteriaceae still persist at 21 months [40]. However, whether the aberrant microbiome and antibiotic resistome of their early lives have any association with lasting consequences of preterm birth warrants further investigation.

NEC as a paradigm of a microbiome dysbiosis-driven disorder

NEC is a devastating pediatric gastrointestinal disorder in preterm infants. Multiple studies now support the concept that a microbial community (and not a specific pathogen) contributes to the pathogenesis of NEC. There is a con-

2.4 Acquisition of the Human Gut Microbiome, continued.

vergence of data demonstrating that microbial dysbiosis occurs prior to NEC onset, as demonstrated by the greater abundance of Gammaproteobacteria (i.e., Gram-negative facultative Bacilli) and lower abundance of strict anaerobic bacteria (especially Negativicutes) in very low birthweight infants (VLBW) [1,2]. *Enterobacteriaceae* including *Klebsiella* [60], *Escherichia*, and *Enterobacter* are repeatedly reported to be over-represented prior to the development of NEC[61].

Studying microbiome-host interaction confers a more complete understanding of pathogenesis of NEC. Recent data demonstrate that maternal IgA, mainly from breast milk, binds to gut *Enterobacteriaceae* and protects against NEC. Greater degrees of IgA-unbound *Enterobacteriaceae* are associated with development of NEC [62]. Future studies should leverage multi-OMICS technology and machine learning that simultaneously incorporate clinical characteristics, the microbiome, immune response, metabolites from maternal milk and infant gut for early and precise diagnosis of NEC.

References

1. Pammi M, Cope J, Tarr PI, et al. Intestinal dysbiosis in preterm infants preceding necrotizing enterocolitis: a systematic review and meta-analysis. *Microbiome*. 2017;5(1):31. doi:10.1186/s40168-017-0248-8
2. Warner BB, Tarr PI. Necrotizing enterocolitis and preterm infant gut bacteria. *Semin Fetal Neonatal Med.* 2016;21(6):394-399. doi:10.1016/j.siny.2016.06.001
3. Stokholm J, Blaser MJ, Thorsen J, et al. Publisher Correction: Maturation of the gut microbiome and risk of asthma in childhood. *Nat Commun*. 2018;9(1):704. doi:10.1038/s41467-018-03150-x
4. Vatanen T, Franzosa EA, Schwager R, et al. The human gut microbiome in early-onset type 1 diabetes from the TEDDY study. *Nature*. 2018;562(7728):589-594. doi:10.1038/s41586-018-0620-2
5. Aagaard K, Ma J, Antony KM, Ganu R, Petrosino J, Versalovic J. The placenta harbors a unique microbiome. *Sci Transl Med*. 2014;6(237):237ra65. doi:10.1126/scitranslmed.3008599
6. Collado MC, Rautava S, Aakko J, Isolauri E, Salminen S. Human gut colonisation may be initiated in utero by distinct microbial communities in the placenta and amniotic fluid. *Sci Rep*. 2016;6:23129. doi:10.1038/srep23129
7. Mshvildadze M, Neu J, Shuster J, Theriaque D, Li N, Mai V. Intestinal microbial ecology in premature infants assessed with non-culture-based techniques. *J Pediatr*. 2010;156(1):20-25. doi:10.1016/j.jpeds.2009.06.063
8. Wilczyńska P, Skarżyńska E, Lisowska-Myjak B. Meconium microbiome as a new source of information about long-term health and disease: questions and answers. *J Matern Fetal Neonatal Med*. 2019;32(4):681-686. doi:10.1080/14767058.2017.1387888
9. de Goffau MC, Lager S, Sovio U, et al. Human placenta has no microbiome but can contain potential pathogens. *Nature*. 2019;572(7769):329-334. doi:10.1038/s41586-019-1451-5
10. Leiby JS, McCormick K, Sherrill-Mix S, et al. Lack of detection of a human placenta microbiome in samples from preterm and term deliveries. *Microbiome*. 2018;6(1):196. doi:10.1186/s40168-018-0575-4
11. Lim ES, Rodriguez C, Holtz LR. Amniotic fluid from healthy term pregnancies does not harbor a detectable microbial community. *Microbiome*. 2018;6(1):87. doi:10.1186/s40168-018-0475-7
12. Leon LJ, Doyle R, Diez-Benavente E, et al. Enrichment of Clinically Relevant Organisms in Spontaneous Preterm-Delivered Placentas and Reagent Contamination across All Clinical Groups in a Large Pregnancy Cohort in the United Kingdom. *Appl Environ Microbiol*. 2018;84(14). doi:10.1128/AEM.00483-18
13. Stinson LF, Keelan JA, Payne MS. Characterization of the bacterial microbiome in first-pass meconium using propidium monoazide (PMA) to exclude nonviable bacterial DNA. *Lett Appl Microbiol.* 2019;68(5):378-385. doi:10.1111/lam.13119

2.4 Acquisition of the Human Gut Microbiome, continued.

14. Chernikova DA, Koestler DC, Hoen AG, et al. Fetal exposures and perinatal influences on the stool microbiota of premature infants. *J Matern Fetal Neonatal Med.* 2016;29(1):99-105. doi:10.3109/14767058.2014.987748
15. DiGiulio DB, Romero R, Amogan HP, et al. Microbial prevalence, diversity and abundance in amniotic fluid during preterm labor: a molecular and culture-based investigation. *PloS One*. 2008;3(8):e3056. doi:10.1371/journal.pone.0003056
16. Stout MJ, Conlon B, Landeau M, et al. Identification of intracellular bacteria in the basal plate of the human placenta in term and preterm gestations. *Am J Obstet Gynecol.* 2013;208(3):226.e1-7. doi:10.1016/j.ajog.2013.01.018
17. Ardissone AN, de la Cruz DM, Davis-Richardson AG, et al. Meconium Microbiome Analysis Identifies Bacteria Correlated with Premature Birth. *PLoS ONE*. 2014;9(3). doi:10.1371/journal.pone.0090784
18. Dornelles LV, Procianoy RS, Roesch LFW, et al. Meconium microbiota predicts clinical early-onset neonatal sepsis in preterm neonates. *J Matern Fetal Neonatal Med*. Published online June 7, 2020:1-9. doi:10.1080/14767058.2020.1774870
19. Prince AL, Ma J, Kannan PS, et al. The placental membrane microbiome is altered among subjects with spontaneous preterm birth with and without chorioamnionitis. *Am J Obstet Gynecol*. 2016;214(5):627.e1-627.e16. doi:10.1016/j.ajog.2016.01.193
20. Romero R, Gomez-Lopez N, Winters AD, et al. Evidence that intra-amniotic infections are often the result of an ascending invasion - a molecular microbiological study. *J Perinat Med*. 2019;47(9):915-931. doi:10.1515/jpm-2019-0297
21. Dominguez-Bello MG, Costello EK, Contreras M, et al. Delivery mode shapes the acquisition and structure of the initial microbiota across multiple body habitats in newborns. *Proc Natl Acad Sci U S A.* 2010;107(26):11971-11975. doi:10.1073/pnas.1002601107
22. Chu DM, Ma J, Prince AL, Antony KM, Seferovic MD, Aagaard KM. Maturation of the infant microbiome community structure and function across multiple body sites and in relation to mode of delivery. *Nat Med.* 2017;23(3):314-326. doi:10.1038/nm.4272
23. Hill CJ, Lynch DB, Murphy K, et al. Evolution of gut microbiota composition from birth to 24 weeks in the INFANTMET Cohort. *Microbiome*. 2017;5(1):4. doi:10.1186/s40168-016-0213-y
24. Azad MB, Konya T, Maughan H, et al. Gut microbiota of healthy Canadian infants: profiles by mode of delivery and infant diet at 4 months. *CMAJ Can Med Assoc J J Assoc Medicale Can.* 2013;185(5):385-394. doi:10.1503/cmaj.121189
25. Tapiainen T, Koivusaari P, Brinkac L, et al. Impact of intrapartum and postnatal antibiotics on the gut microbiome and emergence of antimicrobial resistance in infants. *Sci Rep*. 2019;9(1):10635. doi:10.1038/s41598-019-46964-5
26. Bokulich NA, Chung J, Battaglia T, et al. Antibiotics, birth mode, and diet shape microbiome maturation during early life. *Sci Transl Med.* 2016;8(343):343ra82-343ra82. doi:10.1126/scitranslmed.aad7121
27. Dominguez-Bello MG, De Jesus-Laboy KM, Shen N, et al. Partial restoration of the microbiota of cesarean-born infants via vaginal microbial transfer. *Nat Med.* 2016;22(3):250-253. doi:10.1038/nm.4039
28. Bäckhed F, Roswall J, Peng Y, et al. Dynamics and Stabilization of the Human Gut Microbiome during the First Year of Life. *Cell Host Microbe*. 2015;17(5):690-703. doi:10.1016/j.chom.2015.04.004
29. Jost T, Lacroix C, Braegger CP, Chassard C. New insights in gut microbiota establishment in healthy breast fed neonates. *PloS One*. 2012;7(8):e44595. doi:10.1371/journal.pone.0044595

2.4 Acquisition of the Human Gut Microbiome, continued.

30. Yassour M, Vatanen T, Siljander H, et al. Natural history of the infant gut microbiome and impact of antibiotic treatments on strain-level diversity and stability. *Sci Transl Med*. 2016;8(343):343ra81. doi:10.1126/scitranslmed.aad0917

31. Koenig JE, Spor A, Scalfone N, et al. Succession of microbial consortia in the developing infant gut microbiome. *Proc Natl Acad Sci U S A*. 2011;108 Suppl 1:4578-4585. doi:10.1073/pnas.1000081107

32. Robertson RC, Manges AR, Finlay BB, Prendergast AJ. The Human Microbiome and Child Growth – First 1000 Days and Beyond. *Trends Microbiol*. 2019;27(2):131-147. doi:10.1016/j.tim.2018.09.008

33. Rougé C, Goldenberg O, Ferraris L, et al. Investigation of the intestinal microbiota in preterm infants using different methods. *Anaerobe*. 2010;16(4):362-370. doi:10.1016/j.anaerobe.2010.06.002

34. Jacquot A, Neveu D, Aujoulat F, et al. Dynamics and clinical evolution of bacterial gut microflora in extremely premature patients. *J Pediatr*. 2011;158(3):390-396. doi:10.1016/j.jpeds.2010.09.007

35. Arboleya S, Sánchez B, Milani C, et al. Intestinal microbiota development in preterm neonates and effect of perinatal antibiotics. *J Pediatr*. 2015;166(3):538-544. doi:10.1016/j.jpeds.2014.09.041

36. Cong X, Xu W, Janton S, et al. Gut Microbiome Developmental Patterns in Early Life of Preterm Infants: Impacts of Feeding and Gender. *PloS One*. 2016;11(4):e0152751. doi:10.1371/journal.pone.0152751

37. Hill CJ, Lynch DB, Murphy K, et al. Evolution of gut microbiota composition from birth to 24 weeks in the INFANTMET Cohort. *Microbiome*. 2017;5(1):4. doi:10.1186/s40168-016-0213-y

38. Itani T, Ayoub Moubareck C, Melki I, et al. Establishment and development of the intestinal microbiota of preterm infants in a Lebanese tertiary hospital. *Anaerobe*. 2017;43:4-14. doi:10.1016/j.anaerobe.2016.11.001

39. La Rosa PS, Warner BB, Zhou Y, et al. Patterned progression of bacterial populations in the premature infant gut. *Proc Natl Acad Sci U S A*. 2014;111(34):12522-12527. doi:10.1073/pnas.1409497111

40. Gasparrini AJ, Wang B, Sun X, et al. Persistent metagenomic signatures of early-life hospitalization and antibiotic treatment in the infant gut microbiota and resistome. *Nat Microbiol*. 2019;4(12):2285-2297. doi:10.1038/s41564-019-0550-2

41. Liang G, Zhao C, Zhang H, et al. The stepwise assembly of the neonatal virome is modulated by breastfeeding. *Nature*. 2020;581(7809):470-474. doi:10.1038/s41586-020-2192-1

42. Lim ES, Zhou Y, Zhao G, et al. Early life dynamics of the human gut virome and bacterial microbiome in infants. *Nat Med*. 2015;21(10):1228-1234. doi:10.1038/nm.3950

43. Gasparrini AJ, Crofts TS, Gibson MK, Tarr PI, Warner BB, Dantas G. Antibiotic perturbation of the preterm infant gut microbiome and resistome. *Gut Microbes*. 2016;7(5):443-449. doi:10.1080/19490976.2016.1218584

44. Gibson MK, Wang B, Ahmadi S, et al. Developmental dynamics of the preterm infant gut microbiota and antibiotic resistome. *Nat Microbiol*. 2016;1:16024. doi:10.1038/nmicrobiol.2016.24

45. Martínez I, Maldonado-Gomez MX, Gomes-Neto JC, et al. Experimental evaluation of the importance of colonization history in early-life gut microbiota assembly. Ley RE, Garrett WS, eds. *eLife*. 2018;7:e36521. doi:10.7554/eLife.36521

2.4 Acquisition of the Human Gut Microbiome, continued.

46. Chu DM, Antony KM, Ma J, et al. The early infant gut microbiome varies in association with a maternal high-fat diet. *Genome Med.* 2016;8(1):77. doi:10.1186/s13073-016-0330-z
47. Lundgren SN, Madan JC, Emond JA, et al. Maternal diet during pregnancy is related with the infant stool microbiome in a delivery mode-dependent manner. *Microbiome*. 2018;6(1):109. doi:10.1186/s40168-018-0490-8
48. Savage JH, Lee-Sarwar KA, Sordillo JE, et al. Diet during Pregnancy and Infancy and the Infant Intestinal Microbiome. *J Pediatr.* 2018;203:47-54.e4. doi:10.1016/j.jpeds.2018.07.066
49. O'Neill IJ, Sanchez Gallardo R, Saldova R, et al. Maternal and infant factors that shape neonatal gut colonization by bacteria. *Expert Rev Gastroenterol Hepatol*. Published online June 30, 2020:1-14. doi:10.1080/17474124.2020.1784725
50. Ho NT, Li F, Lee-Sarwar KA, et al. Meta-analysis of effects of exclusive breastfeeding on infant gut microbiota across populations. *Nat Commun*. 2018;9(1):4169. doi:10.1038/s41467-018-06473-x
51. Baumann-Dudenhoeffer AM, D'Souza AW, Tarr PI, Warner BB, Dantas G. Infant diet and maternal gestational weight gain predict early metabolic maturation of gut microbiomes. *Nat Med.* 2018;24(12):1822-1829. doi:10.1038/s41591-018-0216-2
52. Cioffi CC, Tavalire HF, Neiderhiser JM, Bohannan B, Leve LD. History of breastfeeding but not mode of delivery shapes the gut microbiome in childhood. *PloS One.* 2020;15(7):e0235223. doi:10.1371/journal.pone.0235223
53. Cong X, Judge M, Xu W, et al. Influence of Feeding Type on Gut Microbiome Development in Hospitalized Preterm Infants. *Nurs Res.* 2017;66(2):123-133. doi:10.1097/NNR.0000000000000208
54. Ford SL, Lohmann P, Preidis GA, et al. Improved feeding tolerance and growth are linked to increased gut microbial community diversity in very-low-birth-weight infants fed mother's own milk compared with donor breast milk. *Am J Clin Nutr*. 2019;109(4):1088-1097. doi:10.1093/ajcn/nqz006
55. Parra-Llorca A, Gormaz M, Alcántara C, et al. Preterm Gut Microbiome Depending on Feeding Type: Significance of Donor Human Milk. *Front Microbiol.* 2018;9. doi:10.3389/fmicb.2018.01376
56. Gregory KE, Samuel BS, Houghteling P, et al. Influence of maternal breast milk ingestion on acquisition of the intestinal microbiome in preterm infants. *Microbiome*. 2016;4(1):68. doi:10.1186/s40168-016-0214-x
57. Palmer C, Bik EM, DiGiulio DB, Relman DA, Brown PO. Development of the Human Infant Intestinal Microbiota. *PLOS Biol.* 2007;5(7):e177. doi:10.1371/journal.pbio.0050177
58. Tanaka S, Kobayashi T, Songjinda P, et al. Influence of antibiotic exposure in the early postnatal period on the development of intestinal microbiota. *FEMS Immunol Med Microbiol.* 2009;56(1):80-87. doi:10.1111/j.1574-695X.2009.00553.x
59. Moles L, Gómez M, Jiménez E, et al. Preterm infant gut colonization in the neonatal ICU and complete restoration 2 years later. *Clin Microbiol Infect Off Publ Eur Soc Clin Microbiol Infect Dis*. 2015;21(10):936.e1-10. doi:10.1016/j.cmi.2015.06.003
60. Olm MR, Bhattacharya N, Crits-Christoph A, et al. Necrotizing enterocolitis is preceded by increased gut bacterial replication, Klebsiella, and fimbriae-encoding bacteria. *Sci Adv.* 2019;5(12):eaax5727. doi:10.1126/sciadv.aax5727
61. Thänert R, Keen EC, Dantas G, Warner BB, Tarr PI. Necrotizing enterocolitis and the microbiome: Current status and future directions. *Press*.

2.4 Acquisition of the Human Gut Microbiome, continued.

62. Gopalakrishna KP, Macadangdang BR, Rogers MB, et al. Maternal IgA protects against the development of necrotizing enterocolitis in preterm infants. *Nat Med*. 2019;25(7):1110-1115. doi:10.1038/s41591-019-0480-9

2.5 Impact of Diet on Gut Microbes

Miguel A. Valdovinos-Díaz, MD, AGAF

Professor of Gastroenterology. UNAM
Head of Neurogastroenteroly and Motility Unit
Department of Gastroenterology
Instituto Nacional de Ciencias Médicas y Nutrición
Salvador Zubirán
Mexico City, Mexico

Diet is the key element that determines the composition of the human gut microbiome. Both short-term and long-term dietary changes impact the ecology of the microbiota. In the short term, abundant animal protein and animal fat intake, temporarily reduced fiber intake, or the exclusion of gluten or the fermentable oligosaccharides, disaccharides, monosaccharides and polyols (FODMAPs) affect the diversity and relative abundance of beneficial species in the microbiota. Enterotypes are related to long-term dietary habits. The *Bacteroides*-predominant enterotype is associated with diets high in animal proteins and saturated fats, which are more common in the Western world. The *Prevotella*-prevailing enterotype is associated with the high intake of carbohydrates and vegetable fiber that is typical in agrarian societies and vegetarian diets. The *Ruminococcus* enterotype is associated with long-term fruit and vegetable consumption. Even though the relevance of enterotypes is still under discussion, approximately 30 to 40% of the microbiota of adults can change throughout the lifespan and diet is one of the most important factors influencing said change.

Breastfeeding is considered to be the perfect nutrition for infants and young children. During breastfeeding, the gastrointestinal tract is colonized by *Actinobacteria* (*Bifidobacterium breve, B. longum, B. dentium, B. infantis,* and *B. pseudocatenulatum*) and *Firmicutes* (*Lactobacillus, Enterococcus,* and *Clostridium*). An adult-like microbiota is established between 2 and 3 years of age. Its composition will depend on the type of food intake in infancy, adolescence, and adulthood.

Macronutrients and the microbiota

High-carbohydrate diets favor the growth of the families *Lachnospiraceae* and *Ruminococcaceae*, genera *Bacteroides* and *Bifidobacterium*, the species *Clostridium cluster XVIII*, and some enterobacteria. The consumption of fiber containing the carbohydrates now known as microbiota-accessible carbohydrates (MACs), increases the diversity and richness of the gut microbiota, as well as the *Prevotella:Bacteroides* ratio. It also significantly increases several short-chain fatty acid (SCFA) producers, including *Lachnospira, Akkermansia, Bifidobacterium, Lactobacillus, Ruminococcus, Roseburia, Clostridium, Faecalibacterium,* and *Dorea.* High intake of animal fat increases the abundance of bile-tolerant microorganisms, such as *Alistipes, Bilophila*, and *Bacteroides* and reduces the abundance of *Firmicutes*, such as *Roseburia, Eubacterium rectale,* and *Ruminococcus bromii*, which metabolize plant polysaccharides. High protein diets increase the abundance of *Roseburia, Eubacterium rectale, Faecalbacterium prausnitzii, Lactobacillus*, and *Bacteroides.* Dietary macronutrients can also impact other microorganisms, such as archaea, fungi, and bacteriophages. Carbohydrate consumption has been associated with a greater abundance of *Methanobrevibacter*, an archaean that increases the production of methane and SCFAs by metabolizing hydrogen. Diet can have an influence on fungal communities, which have been associated with the pathogenesis of inflammatory bowel disease (IBD).

Diet and microbial metabolites

Diets impact the composition of the microbiota, but the effect they produce on the gut metabolome is more relevant. Dietary fiber is metabolized by the gut microbiota to produce acetate, propionate, and butyrate, which are the SCFAs that regulate immune function and gut hormone production and maintain gut barrier function, lipogenesis, blood-brain barrier integrity, and brain function. Proteins and amino acids are deaminated by the gut microbiota to produce SCFAs, branched-chain amino acids (isobutyrate, isovalerate and 2-methylbutyrate), phenol compounds (phenylpropionate, phenylacetate, p-cresol, indole propionate and indole acetate), amines, sulfides, and ammonia. Those products are beneficial for the host, but others have been associated with disease. The protein in red meat is a

2.5 Impact of Diet on Gut Microbes, continued.

source of L-carnitine, whose bacterial catabolism results in the formation of trimethylamine (TMA). TMA is metabolized by the liver into trimethylamine oxide (TMAO), a molecule that is strongly associated with the risk for coronary vascular disease because it promotes the development of atherosclerosis. Diets rich in fat are associated with lower SCFA production and an increase in the bile acids that reach the colon and are dehydroxylated by the microbiota into carcinogenic secondary bile acids.

Food additives

Over the past few decades, one of the most significant human dietary changes has been the consumption of ultra-processed foods, which contain natural or synthetic additives, such as the non-caloric artificial sweeteners (NASs) and emulsifiers approved for alimentary use in the food industry. NAS consumption can alter the gut microbiota and induce microbiota-mediated adverse effects in the host, such as glucose intolerance and metabolic syndrome. Studies have shown that NAS intake increases the abundance of *Bacteroides* and some *Clostridiales spp*. and reduces the abundance of *Bifidobacterium* and *Lactobacillus*. In contrast, steviol glycosides (extracted from the stevia leaf) have not been associated with significant changes in the gut microbiota. The impact of NASs on the gut microbiota and energy metabolism requires further investigation.

Carboxymethyl-cellulose, polysorbate 80, lecithin, and mono- and diglycerides of fatty acids are the most commonly used food additive emulsifiers. In epidemiologic studies, the elevated consumption of those agents has been associated with cardiovascular and metabolic disorders and Crohn's disease. Emulsifiers have effects on the gut microbiota, mucosal barrier, and inflammatory pathways and can induce disease in animal models, but there is no evidence directly linking emulsifiers to human disease.

Dietary patterns

Various popular diets, including the Mediterranean diet, ketogenic diet, vegetarian diet, vegan diet, gluten-free diet, low FODMAP diet, and intermittent fasting, have been adopted to preserve health or to achieve different therapeutic aims. Some of those dietary patterns have been evaluated for their ability to modulate the gut microbiota. A limitation in the study of those diets is the fact that the experimental manipulation of a specific nutrient invariably modifies the intake of some other macronutrient. Therefore, a large part of dietary evidence has been acquired from experiments on animals.

The Mediterranean diet

The Mediterranean diet consists of a predominant consumption of fruits, vegetables, legumes, non-saturated fats, fish, olive oil, and wine, and a limited consumption of meat. Several epidemiologic studies have shown that the Mediterranean diet reduces the risk of all-cause mortality and multiple chronic diseases. Good adherence to the diet has been associated with lower *Firmicutes:Bacteroidetes* ratios and higher levels of fecal SCFAs.

The ketogenic diet

The ketogenic diet is a low-carbohydrate diet (5 to 10% of caloric intake) for increasing ketone production. It was originally developed to control refractory epilepsy in children, but in the last few years, it has been adopted for weight reduction, as well as for other neurologic disorders. Studies on humans have shown a negative impact on the ecology of the gut microbiota, with a decrease in its overall richness. In children with epilepsy, the ketogenic diet has shown a reduction in *Bifidobacteria, E. rectale*, and *Dialister* and an increase in the relative abundance of *Actinobacteria* and *Escherichia coli.*

Vegetarian/Vegan diets

Plant-rich diets have been associated with positive health outcomes and reduced disease risk. Studies that have compared omnivore diets with vegetarian diets have shown modest differences in the diversity and richness of the gut microbiota and a greater effect at the genus and species levels. Vegans have higher counts of certain *Bacteroidetes*, particularly *Prevotella*, compared with omnivores. The fecal levels of SCFAs positively correlate with the consumption of fruits, vegetables, and legumes.

Intermittent fasting

Clinical trials on adults with overweight have shown that intermittent fasting is beneficial in different conditions,

2.5 Impact of Diet on Gut Microbes, continued.

such as obesity, diabetes mellitus, cardiovascular disease, cancer, and neurologic diseases. Studies on humans have shown that fasting interventions increase the abundance of *Faecalibacterium prausnitzii, A. muciniphila,* and species of bifidobacteria. The association between those changes in the ecology of the microbiota and the metabolic benefits of intermittent fasting has yet to be demonstrated.

The gluten-free diet

The gluten-free diet (GFD) impacts the gut microbiota of healthy subjects. Different studies have shown an increase in the abundance of *E. coli, Slackia, Victivallaceae, Enterobacteriaceae, Clostridiaceae, Coriobacteriaceae,* an unclassified species of *Clostridiales*, and *Lachnospiraceae* and a decrease in *C. lituseburense, Lactobacillus, F. prausnitzii, Bifidobacterium spp., Dorea, B. wexlerae, A. hadrus, E. halli, Veillonellaceae, R. bromii*, and *R. faecis.* A relative abundance of *Proteobacteria* and a reduction of *Bacteroidetes* and *Firmicutes* have been observed in patients with celiac disease (CD) that persist with gastrointestinal symptoms, despite being on a GFD. In patients not presenting with active disease, there was a decrease in the abundance of *Bifidobacteria* in the gut microbiota. An increase in *Pseudomonas* species has also been documented in 50% of patients with CD. On the other hand, in patients with nonceliac gluten/wheat sensitivity (NCG/WS) that are on a GFD, there is an abundance of *Bacteroidaceae*, *Roseburia, F. prausnitzii* and *Pseudomonas* species and a decrease in *Lachnospiraceae*, *Bacteroides, Blautia, Dorea, Coprococcus*, and *Collinsella* in the fecal microbiota. Those findings suggest that a GFD in healthy subjects both reduces the bacterial richness of the gut microbiota and impacts its composition differently from patients with CD or NCG/WS. In healthy subjects, a GFD causes a depletion of beneficial species, e.g., *Bifidobacteria*, and favors the growth of opportunistic pathogens, such as *Enterobacteriaceae* and *E. coli.* In contrast, in patients with CD or NCG/WS, a GDF can restore the gut microbiota, reducing proinflammatory species and improving gastrointestinal symptoms.

The low FODMAP diet

FODMAPs induce abdominal symptoms in patients with irritable bowel syndrome (IBS). The mechanisms through which FODMAPs cause symptoms in IBS include increased water in the small bowel, due to the osmotic effect, and increased gas in the colon, due to bacterial fermentation. A low FODMAP diet provides symptomatic relief in 50 to 80% of patients with IBS, particularly improving bloating, flatulence, diarrhea, and overall symptoms.

FODMAP carbohydrates include fructo-oligosaccharides (FOSs), galacto-oligosaccharides (GOSs), xylo-oligosaccharides (XOSs), polyols, and fructose, which have prebiotic action and stimulate the growth of bacteria that are beneficial for health, such as *Bifidobacteria, Akkermansia muciniphila*, and *Faecalibacterium prausnitzii.* In contrast, the low FODMAP diet negatively impacts the ecology of the gut microbiota. The majority of studies have shown that a low FODMAP diet does not alter the richness and alpha diversity of the microbiota. In all the studies, the relative abundance of the phylum *Actinobacteria* and the genus *Bifidobacterium* are reduced during low FODMAP intake. In addition, low FODMAP diet reduces the abundance of butyrate-producing bacteria in the phylum *Firmicutes,* particularly *F. prausnitzii*, and increases the relative abundance of the genus *Bilophila*. Those changes can be reversed through FOS, GOS, and inulin supplementation.

Precision nutrition

The aim of precision nutrition is to identify key characteristics of the microbiome in an individual to predict the response to specific food components, so that a diet resulting in positive outcomes can be designed. There is evidence that particular species of the gut microbiota can be predictors of the response to a specific diet. Subjects that present with improved glucose metabolism after barley kernel-based bread consumption have been associated with having a greater abundance of *Prevotella* in their gut microbiota. In adults with overweight and obesity, those with a baseline abundance of *Akkermansia muciniphila* showed significant improvement in insulin sensitivity and lipid metabolism, as well as a greater reduction in body fat, after a low-calorie diet. In children with IBS, those that responded to a low FODMAP diet had a high proportion of *Bacteroidaceae, Erysipelotrichaceae*, and Clostridiales species.

2.5 Impact of Diet on Gut Microbes, continued.

More exact methods for predicting the response to diet have been developed, through the combination of baseline microbiome signatures with other individual traits. In a study on overweight or obese nondiabetic individuals, interpersonal variability in the postprandial glycemic response to identical foods was predicted by the gut microbiome, dietary habits, anthropometrics, and blood parameters, using a machine-learning approach. Those findings suggest that the use of precision nutrition is necessary for achieving the predictive results of a particular diet in different individuals.

Conclusions

Diet significantly modifies the ecology of the gut microbiome, which in turn, has a profound impact on health and disease. There have been important advances in the knowledge of the beneficial or harmful effects of different popular dietary patterns and food additives on the gut microbiome and metabolome. Without a doubt, diet is probably the most powerful tool available for gut microbiome modulation, but a greater understanding of the intricate diet-host-microbiota interactions is still needed. Precision nutrition is beginning to be utilized in clinical practice for predicting the response to a specific diet and improving therapeutic outcomes.

FOR FURTHER READING

1. Caio G, et al. Effect of Gluten-Free Diet on Gut Microbiota Composition in Patients with Celiac Disease and Non-Celiac Gluten/Wheat Sensitivity. Nutrients. 2020;12(6):1832.
2. Costea PI, et al. Enterotypes in the landscape of gut microbial community composition Nat Microbiol. 2018;3(1):8-16.
3. Gibson PR, et al. Review article: FODMAPS, prebiotics and gut health-the FODMAP hypothesis revisited. Aliment Pharmacol Ther. 2020;52(2):233-246.
4. Halmos EP, et al. Review article: emulsifiers in the food supply and implications for gastrointestinal disease. Aliment Pharmacol Ther. 2019;49(1):41-50.
5. Kolodziejczyk AA, et al. Diet-microbiota interactions and personalized nutrition. Nat Rev Microbiol. 2019;17(12):742-753.
6. Quigley EMM, et al. Recent advances in modulating the microbiome. F1000Res. 2020;9:F1000 Faculty Rev-46.
7. Zmora N, et al. You are what you eat: diet, health and the gut microbiota. Nat Rev Gastroenterol Hepatol. 2019;16(1):35-56.

3.1 Gut Microbiota in Functional Bowel Disorders

Anusha S Thomas, MD

Department of Gastroenterology, Hepatology and Nutrition
MD Anderson Cancer Center
Houston, Texas, USA

Eamonn M M Quigley, MD, FRCP, FACP, MACG, FRCPI, MWGO

Lynda K and David M Underwood Center for Digestive Disorders
Houston Methodist Hospital and
Weill Cornell Medical College
Houston, Texas, USA

Alexander C Ford, MD, FRCP

Leeds Gastroenterology Institute
Leeds Institute of Medical Research at St James' Hospital
University of Leeds
Leeds, United Kingdom

Irritable bowel syndrome (IBS) and functional (or chronic idiopathic) constipation (FC) are two of the most prevalent and best studied functional bowel disorders in the Western world[1]. Although non-fatal, they impact on quality of life, personal relationships and productivity and impose a significant socioeconomic burden on the individual as well as on society at large. Formerly regraded as distinct entities, it is now recognized that FC and the constipated variety of IBS, IBS with constipation (IBS-C), form part of a spectrum, with abdominal pain being a more dominant feature in IBS-C.

Rome IV criteria for the diagnosis of IBS require that patients have had recurrent abdominal pain on an average of at least 1 day per week during the previous 3 months that is associated with two or more of the following [1]:

- Related to defecation (may be increased or unchanged by defecation)
- Associated with a change in stool frequency
- Associated with a change in stool form or appearance

Patients with FC should not meet IBS criteria, although abdominal pain and/or bloating may be present but are not predominant symptoms. Symptom onset should occur at least 6 months before diagnosis, and symptoms should be present during the last 3 months. Symptoms must include two or more of the following:

- Straining during more than one-fourth (25%) of defecations
- Lumpy or hard stools (rated 1 or 2 on the Bristol Scale - BSFS) more than one-fourth (25%) of defecations
- Sensation of incomplete evacuation more than one-fourth (25%) of defecations
- Sensation of anorectal obstruction/blockage more than one-fourth (25%) of defecations
- Manual maneuvers to facilitate more than one fourth (25%) of defecations (e.g., digital evacuation, support of the pelvic floor)
- Fewer than 3 spontaneous bowel movements per week

IBS

Although the pathogenesis of IBS is certainly multifactorial, the concept of the gut-brain has served as a useful paradigm to explain IBS symptoms with dysfunction at various points along the axis from cortex to gut muscle, nerve and mucosa variably contributing to presentation in different individuals. Phenomena encompassed within this framework include visceral hypersensitivity, altered gut motility, central hypervigilance and accentuated stress responses. Other factors such genetic predisposition, psychological distress and neurohormonal impacts can interact to generate the very varied phenotype that typifies IBS, as well as the variable severity of its symptomatology[3-6].

3.1 Gut Microbiota in Functional Bowel Disorders, continued.

The gut microbiome and IBS

Most recently, connections between the gut and the brain have been extended to include a new participant; the microbiome, leading to the concept of the microbiome-gut-brain axis. Indeed, tantalizing evidence has emerged, primarily, it must be conceded, from animal models to suggest that bacteria resident in the gut could impact on the "big brain" and even contribute to neurological and neuropsychiatric disease. Accordingly, the microbiota has emerged as a potential therapeutic target in disorders as diverse as IBS, Parkinson's disease (PD) and depression[7]. There is substantial experimental data to indicate the ability of gut microbes to influence components of the gut barrier, the intestinal immune system and the neuromuscular apparatus of the gastrointestinal tract, as well as central nervous system structure and function[8-11]. Indeed, colonization of germ-free mice with feces from patients with IBS has been shown to alter transit and induce visceral hypersensitivity[12,13].

That the microbiota might be a factor in IBS was first suggested by the observation that IBS could develop *de novo* in the aftermath of acute enteric bacterial, viral or parasitic infections[14]. More recently, modern sequencing technology has been applied to the study of the fecal and colonic microbiota in IBS, in general, and relationships between a variety of clinical and demographic parameters and the microbiota investigated. Although data remains limited, and not always consistent, it is evident that IBS patients have an altered fecal microbiota relative to healthy individuals[15]. Currently available data are fraught with challenges in interpretation – small study populations, variations in patient selection and methodology not to mention a failure to account for such confounders as diet, therapy, co-morbid psychopathology and symptom severity. Nonetheless, some overall patterns have emerged: the fecal and colonic mucosal microbiota are different in IBS and the fecal microbiota may not only predict severity[16] but also responsiveness to one common intervention – the low fermentable oligo-, di- and monosaccharides and polyols (FODMAP) diet[17]. It is now abundantly clear that the expectation that a single microbial signature might typify IBS was very naïve; the heterogeneity of its symptoms and severity, as well as the impact of diet, sex, medications and other factors should have prepared us for a far from definitive answer. However, recent data derived in a longitudinal study that employed a multi-omics approach was able to identify IBS subtype-specific and symptom-related variations in microbial composition and function, and were also able to relate certain bacterial metabolites with physiological mechanisms relevant to IBS in the host[18]. A disturbed microbiome or an aberrant host response to the microbiome might well involve the generation of intraluminal molecules with biological effects on motility, sensation, gut barrier function, immune activation and, of course, communication with the central nervous system.

Small intestinal bacteria might also play a role: although its precise prevalence and role in IBS remain uncertain, evidence has been presented to associate small intestinal bacterial overgrowth (SIBO) with IBS[19]. The diagnosis of SIBO has been fraught with the methodological limitations inherent to our current diagnostic armamentarium: jejunal aspiration and breath tests[20]. In the most detailed investigation to date, the small intestinal microbiome was shown to be altered in IBS but SIBO, as conventionally defined, was not relevant to clinical presentation[21]. Exciting as the concept of the microbiome-gut-brain axis may appear, we are still a long way from understanding its precise role in the genesis of symptoms in IBS.

Meanwhile, the most clinically compelling evidence for a role of gut bacteria in IBS comes from clinical trials relating to interventions that modulate the microbiome, including diet, antibiotics, prebiotics, synbiotics, probiotics and fecal microbiota transplantation/transfer in IBS[22,23]. For example, the poorly absorbed antibiotic rifaximin, and synbiotics and probiotics, in general, have been shown to ameliorate the cardinal symptoms of IBS. Quite how these interventions achieve their effects in IBS is unclear.

Diet and microbiome and IBS:

Fiber has been one of the most time-honored dietary intervention in IBS. Fermentable, soluble fibers (synthetic and natural) increase stool frequency, improve stool consistency and accelerate transit; thereby, serving as attractive options in the therapy of constipation- predominant IBS. Fiber serves as a key substrate for gut microbiota to produce the short chain fatty acids (SCFAs) acetate, propionate

3.1 Gut Microbiota in Functional Bowel Disorders, continued.

and butyrate which, in turn, provide an energy source for colonocytes and serve to regulate gut integrity, immunity and permeability[24].

A diet low in FODMAPs has achieved widespread popularity in the management of IBS, with efficacy supported by clinical trials[25]. FODMAPs are small, osmotically active carbohydrates that are poorly absorbed in the small intestine and rapidly fermented by colonic microbiota to release gas and SCFAs, leading to an exacerbation of IBS symptoms such as abdominal pain and bloating. Not surprisingly, a low FODMAP diet impacts on the colonic microbiome, leading to the depletion of some important commensals[26]; the long-term consequences of this finding are unknown at this time.

Prebiotics, Probiotics, Postbiotics and the microbiome in IBS

Huaman and colleagues compared a low FODMAP diet with a prebiotic in the form of beta-galacto-oligosaccharide (GOS) in a randomized controlled trial in patients with functional gastrointestinal disorders and flatulence[27]. As expected, the low FODMAP diet decreased, while the prebiotic increased, the abundance of bifidobacteria; the exact reverse effects were seen in relation to *Bilophila wadsworthia*[27]. Both strategies reduced symptoms to an equally significant extent, with the exception of flatulence and borborygmi whose reductions did not achieve statistical significance in the group administered the prebiotic; indeed, the prebiotic did not exacerbate any of these supposedly "gas-related" symptoms. The explanation for these reassuring observations is provided by a separate study, again from the same group, which demonstrated that, over a period as short as 2 weeks, the microbiota adapts to GOS administration by shifting to low gas-producing pathways[28].

With regard to probiotics there have been multiple studies in IBS and the accumulated data does indicate a beneficial effect[22]; differences in study design, probiotic strain, dose and formulation, as well as study population and outcomes, render the selection of the optimal strain(s) nigh impossible. Experimental data supports the plausibility of a probiotic effect in IBS, given that impacts on gut-brain signaling, motility, visceral sensation, the gut barrier and the mucosal and systemic inflammatory responses have been demonstrated in a variety of animal models[29]. Limited data from humans indicates that certain probiotics can impact on brain function[30-32].

An alternative approach to "bacteriotherapy" involves the use of dead or inactivated bacteria, bacterial components or their products; so-called post-biotics. This approach has a number of practical and commercial advantages and, very recently, a formulation featuring heat-inactivated *B. bifidum* HI-MIMBb75 was shown to substantially alleviate IBS and its symptoms in what the authors referred to as "a real-life setting"[33].

Antibiotics and IBS

Rifaximin has been shown to be effective in non-constipated IBS with a short 14-day course inducing prolonged remission in some responders; retreatment is also effective[34]. The rifaximin story may be more complicated than it would appear at first sight – its effects in IBS may not be dependent on the eradication of SIBO or on the modulation of bacterial populations[34].

Fecal microbiota transplantation and IBS

Based on the assumption that gut microbial communities are disturbed ("dysbiotic") in IBS and aware of the success and overall excellent safety record of fecal microbiota transplantation/transfer (FMT) in the management of severe or recurrent *Clostridioides difficile* infection, and in the context of a relatively poor impact of available therapies, it should come as no surprise that FMT has been employed in IBS[36-46]. Results to date have been somewhat mixed with a number of studies showing benefit[36,38,40,42,43], while others have not[37,39,41]. Success has been variably attributed to dose, method of delivery, the nature of the recipient population and the composition of the donated material but remains to be clearly defined. It does appear that favorable responses are associated with certain changes in the recipient microbiome and SCFA production[36,44-46].

FC

In comparison with IBS, studies on microbiota composition in FC are scanty[47-50]; a coherent pattern has yet to emerge. In addition to the multiple confounding factors outline above in relation to IBS, studies of microbiota in FC

3.1 Gut Microbiota in Functional Bowel Disorders, continued.

have to contend with an additional factor – the impact of slowed colonic transit and resultant fecal stasis[47,49]; does an altered microbiota alter motility or does delayed colon transit change the composition of the colonic microbiome? Fiber is a time-honored and effective treatment for constipation, regardless of cause[51], and has been known for decades to alter the density of colonic bacteria[52,53]. The relative primacy of these proliferative effects on bacteria over other effects of fiber and prebiotics remains to be defined. Again, in comparison with IBS, studies of other interventions that modulate colonic microbiota have been relatively few in FC. There are a number of microbe-mechanisms whereby probiotics or FMT could ameliorate constipation-related symptoms (e.g. via SCFA production or bile salt metabolism)[54,55]. For now, however, evidence for clinical efficacy of probiotics in FC is weak and requires further study[56,57].

Conclusions

It should come as no surprise, given advances in techniques to study the microbiota coupled with exciting data from animal models, that the paradigm of the microbiota-gut-brain axis has been proposed as relevant to IBS. To many it will be seen as a logical extension of that model that has become so central to our understanding of IBS – the gut-brain axis. The possibility that a disturbed microbiome, or an aberrant host-response to that same microbiome, might be relevant to IBS and could impact on the CNS is now being contemplated seriously and investigated, and has the potential to open new diagnostic and therapeutic vistas on this challenging disorder. As much of the extant data comes from animal models one must remain cautious in their interpretation – no single animal model can recapitulate the IBS phenotype. The bi-directionality of microbiota-gut-brain interactions must also be remembered – the complex interactions between inflammation and the gut microbiota exemplify how a disease state can impact on the microbiota. With regard to interventions, there are many intriguing approaches, but there seems some way to go to personalized pharmabiotic therapy for that very special individual – the IBS sufferer.

References

1. Sperber AD, Bangdiwala SI, Drossman DA, et al. Worldwide Prevalence and Burden of Functional Gastrointestinal Disorders, Results of Rome Foundation Global Study. Gastroenterology 2020 [epub ahead of print].
2. Lacy BE, Mearin F, Change L, et al. Bowel Disorders. Gastroenterology 2016;150:1393-1407.
3. Camilleri M, Di Lorenzo C. Brain-gut axis: from basic understanding to treatment of IBS and related disorders. J Pediatr Gastroenterol Nutr. 2012;54:446-53.
4. Saito YA. Genes and irritable bowel syndrome: is there a link? Curr Gastroenterol Rep. 2008;10:355-62.
5. Camilleri M. Physiological underpinnings of irritable bowel syndrome: neurohormonal mechanisms. J Physiol. 2014;592:2967-80.
6. Drossman DA, Chang L, Bellamy N, et al. Severity in irritable bowel syndrome: a Rome Foundation Working Team report. Am J Gastroenterol. 2011;106:1749-59.
7. Quigley EMM. Microbiota-Brain-Gut Axis and Neurodegenerative Diseases. Curr Neurol Neurosci Rep 2017;17:94.
8. Neish AS, Gewirtz AT, Zeng H, Young AN, Hobert ME, Karmali V, Rao AS, Madara JL. Prokaryotic regulation of epithelial responses by inhibition of IkappaB-alpha ubiquitination. Science 2000;289:1560-3.
9. Dey N, Wagner VE, Blanton LV, et al. Regulators of gut motility revealed by a gnotobiotic model of diet-microbiome interactions related to travel. Cell 2015;163:95-107.
10. Savidge TC. Epigenetic Regulation of Enteric Neurotransmission by Gut Bacteria. Front Cell Neurosci. 20169:503.
11. Mayer EA, Tillisch K, Gupta A. Gut-brain axis and the microbiota. J Clin Invest. 2015;125:926-38.

3.1 Gut Microbiota in Functional Bowel Disorders, continued.

12. Kashyap PC, Marcobal A, Ursell LK, et al. Complex interactions among diet, gastrointestinal transit, and gut microbiota in humanized mice. Gastroenterology. 2013;144:967-77.
13. Crouzet L, Gaultier E, Del'Homme C, et al. The hypersensitivity to colonic distension of IBS patients can be transferred to rats through their fecal microbiota. Neurogastroenterol Motil. 2013;25:e272-82.
14. Klem F, Wadhwa A, Prokop LJ, et al. Prevalence, Risk Factors, and Outcomes of Irritable Bowel Syndrome After Infectious Enteritis: A Systematic Review and Meta-analysis. Gastroenterology. 2017;152:1042-1054.
15. Pittayanon R, Lau JT, Yuan Y, et al. Gut Microbiota in Patients With Irritable Bowel Syndrome-A Systematic Review. Gastroenterology. 2019;157:97-108.
16. Tap J, Derrien M, Törnblom H, et al. Identification of an Intestinal Microbiota Signature Associated With Severity of Irritable Bowel Syndrome. Gastroenterology. 2017;152:111-123.
17. Bennet SMP, Böhn L, Störsrud S, et al. Multivariate modelling of faecal bacterial profiles of patients with IBS predicts responsiveness to a diet low in FODMAPs. Gut 2018;67:872-81.
18. Mars RAT, Yang Y, Ward T, et al. Longitudinal Multi-omics Reveals Subset-Specific Mechanisms Underlying Irritable Bowel Syndrome. Cell. 2020;183:1137-1140.
19. Ford AC, Spiegel BM, Talley NJ, Moayyedi P. Small intestinal bacterial overgrowth in irritable bowel syndrome: systematic review and meta-analysis. Clin Gastroenterol Hepatol. 2009;7:1279-86.
20. Quigley EMM, Murray JA, Pimentel M. AGA Clinical Practice Update on Small Intestinal Bacterial Overgrowth: Expert Review. Gastroenterology. 2020;159:1526-1532.
21. Saffouri GB, Shields-Cutler RR, Chen J, et al. Small intestinal microbial dysbiosis underlies symptoms associated with functional gastrointestinal disorders. Nat Commun. 2019;10:2012.
22. Ford AC, Harris LA, Lacy BE, Quigley EMM, Moayyedi P. Systematic review with meta-analysis: the efficacy of prebiotics, probiotics, synbiotics and antibiotics in irritable bowel syndrome. Aliment Pharmacol Ther. 2018;48:1044-60.
23. Myneedu K, Deoker A, Schmulson MJ, Bashashati M. Fecal microbiota transplantation in irritable bowel syndrome: A systematic review and meta-analysis. United European Gastroenterol J. 2019;7:1033-1041.
24. Koh A, DeVadder F, Kovatcheva-Datchary P, Bäckhed F. From dietary fiber to host physiology: Short-chain fatty acids as key bacterial metabolites. Cell 2016;165:1332–1345.
25. Dionne J, Ford AC, Yuan Y, et al. Systematic Review and Meta-Analysis Evaluating the Efficacy of a Gluten-Free Diet and a Low FODMAPs Diet in Treating Symptoms of Irritable Bowel Syndrome. Am J Gastroenterol. 2018;113:1290-1300.
26. Staudacher HM, Lomer MCE, Farquharson FM, et al. A Diet Low in FODMAPs Reduces Symptoms in Patients With Irritable Bowel Syndrome and A Probiotic Restores Bifidobacterium Species: A Randomized Controlled Trial. Gastroenterology 2017;153, 936–947.
27. Huaman JW, Mego M, Manichanh C, et al. Effects of Prebiotics vs a Diet Low in FODMAPs in Patients With Functional Gut Disorders. Gastroenterology. 2018;155:1004-1007.
28. Mego M, Accarino A, Tzortzis G, et al. Colonic gas homeostasis: Mechanisms of adaptation following HOST-G904 galactooligosaccharide use in humans. Neurogastroenterol Motil. 2017;29(9).
29. Quigley EM. Probiotics in Irritable Bowel Syndrome: The Science and the Evidence. J Clin Gastroenterol. 2015;49 Suppl 1:S60-4.
30. Tillisch K, Labus J, Kilpatrick L, et al. Consumption of fermented milk product with probiotic modulates brain activity. Gastroenterology. 2013;144:1394-401.

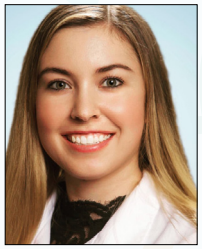
3.1 Gut Microbiota in Functional Bowel Disorders, continued.

31. Pinto-Sanchez MI, Hall GB, Ghajar K, et al. Probiotic Bifidobacterium longum NCC3001 Reduces Depression Scores and Alters Brain Activity: A Pilot Study in Patients With Irritable Bowel Syndrome. Gastroenterology. 2017;153:448-459.
32. Wang H, Braun C, Murphy EF, Enck P. Bifidobacterium longum 1714™ Strain Modulates Brain Activity of Healthy Volunteers During Social Stress. Am J Gastroenterol. 2019;114:1152-1162.
33. Andresen V, Gschossmann J, Layer P. Heat-inactivated Bifidobacterium bifidum MIMBb75 (SYN-HI-001) in the treatment of irritable bowel syndrome: a multicentre, randomised, double-blind, placebo-controlled clinical trial. Lancet Gastroenterol Hepatol. 20205:658-666.
34. Lembo A, Pimentel M, Rao SS, et al. Repeat Treatment With Rifaximin Is Safe and Effective in Patients With Diarrhea-Predominant Irritable Bowel Syndrome. Gastroenterology. 2016;151:1113-1121.
35. Fodor AA, Pimentel M, Chey WD, et al. Rifaximin is associated with modest, transient decreases in multiple taxa in the gut microbiota of patients with diarrhoea-predominant irritable bowel syndrome. Gut Microbes. 2019;10:22-33.
36. Mizuno S, Masaoka T, Naganuma M, et al. Mizuno S, et al. Digestion 2017;96:29-38. Bifidobacterium-Rich Fecal Donor May Be a Positive Predictor for Successful Fecal Microbiota Transplantation in Patients with Irritable Bowel Syndrome. Digestion. 2017;96:29-38.
37. Halkjær SI, Christensen AH, Lo BZS, et al. Faecal microbiota transplantation alters gut microbiota in patients with irritable bowel syndrome: results from a randomised, double-blind placebo-controlled study. Gut. 2018;67:2107-2115.
38. Johnsen PH, Hilpüsch F, Cavanagh JP, et al. Faecal microbiota transplantation versus placebo for moderate-to-severe irritable bowel syndrome: a double-blind, randomised, placebo-controlled, parallel-group, single-centre trial. Lancet Gastroenterol Hepatol. 2018;3:17-24.
39. Aroniadis OC, Brandt LJ, Oneto C, et al. Faecal microbiota transplantation for diarrhoea-predominant irritable bowel syndrome: a double-blind, randomised, placebo-controlled trial. Lancet Gastroenterol Hepatol. 2019;4:675-685.
40. Johnsen PH, Hilpüsch F, Valle PC, Goll R. The effect of fecal microbiota transplantation on IBS related quality of life and fatigue in moderate to severe non-constipated irritable bowel: Secondary endpoints of a double blind, randomized, placebo-controlled trial. EBioMedicine. 2020;51:102562.
41. Lahtinen P, Jalanka J, Hartikainen A, et al. Randomised clinical trial: faecal microbiota transplantation versus autologous placebo administered via colonoscopy in irritable bowel syndrome. Aliment Pharmacol Ther. 2020;51:1321-1331.
42. El-Salhy M, Hatlebakk JG, Gilja OH, Bråthen Kristoffersen A, Hausken T. Efficacy of faecal microbiota transplantation for patients with irritable bowel syndrome in a randomised, double-blind, placebo-controlled study. Gut. 2020;69(5):859-867.
43. Holvoet T, Joossens M, Vázquez-Castellanos JF, et al. Fecal Microbiota Transplantation Reduces Symptoms in Some Patients With Irritable Bowel Syndrome With Predominant Abdominal Bloating: Short- and Long-term Results From a Placebo-Controlled Randomized Trial. Gastroenterology. 2020 [epub ahead of print].
44. Mazzawi T, Hausken T, Hov JR, et al. Clinical response to fecal microbiota transplantation in patients with diarrhea-predominant irritable bowel syndrome is associated with normalization of fecal microbiota composition and short-chain fatty acid levels. Scand J Gastroenterol. 2019;54:690-699.
45. Goll R, Johnsen PH, Hjerde E, et al. Effects of fecal microbiota transplantation in subjects with irritable bowel syndrome are mirrored by changes in gut microbiome. Gut Microbes. 2020;12:1794263.

3.1 Gut Microbiota in Functional Bowel Disorders, continued.

46. El-Salhy M, Valeur J, Hausken T, Gunnar Hatlebakk J. Changes in fecal short-chain fatty acids following fecal microbiota transplantation in patients with irritable bowel syndrome. Neurogastroenterol Motil. 2020:e13983.
47. Yarullina DR, Shafigullin MU, Sakulin KA, et al. Characterization of gut contractility and microbiota in patients with severe chronic constipation. PLoS One. 2020;15:e0235985.
48. Chen YR, Zheng HM, Zhang GX, et al. High Oscillospira abundance indicates constipation and low BMI in the Guangdong Gut Microbiome Project. Sci Rep. 2020;10:9364.
49. Müller M, Hermes GDA, Canfora EE, et al. Distal colonic transit is linked to gut microbiota diversity and microbial fermentation in humans with slow colonic transit. Am J Physiol Gastrointest Liver Physiol. 2020;318:G361-G369.
50. Tian H, Chen Q, Yang B, et al. Analysis of Gut Microbiome and Metabolite Characteristics in Patients with Slow Transit Constipation. Dig Dis Sci. 2020 [epub ahead of print].
51. Christodoulides S, Dimidi E, Fragkos KC, et al. Systematic review with meta-analysis: effect of fibre supplementation on chronic idiopathic constipation in adults. Aliment Pharmacol Ther. 2016;44:103-16.
52. Müller M, Hermes GDA, Emanuel EC, et al. Effect of wheat bran derived prebiotic supplementation on gastrointestinal transit, gut microbiota, and metabolic health: a randomized controlled trial in healthy adults with a slow gut transit. Gut Microbes. 2020;12:1704141.
53. Fu X, Li R, Zhang T, Li M, Mou H. Study on the ability of partially hydrolyzed guar gum to modulate the gut microbiota and relieve constipation. J Food Biochem. 2019;43:e12715.
54. Dimidi E, Mark Scott S, Whelan K. Probiotics and constipation: mechanisms of action, evidence for effectiveness and utilisation by patients and healthcare professionals. Proc Nutr Soc. 2020;79:147-157.
55. Botelho PB, Ferreira MVR, Araújo AM, et al. Effect of multispecies probiotic on gut microbiota composition in individuals with intestinal constipation: A double-blind, placebo-controlled randomized trial. Nutrition. 2020;78:110890.
56. Wen Y, Li J, Long Q, Yue CC, et al. The efficacy and safety of probiotics for patients with constipation-predominant irritable bowel syndrome: A systematic review and meta-analysis based on seventeen randomized controlled trials. Int J Surg. 2020;79:111-119.
57. Kamiński M, Skonieczna-Żydecka K, Łoniewski I, et al. Are probiotics useful in the treatment of chronic idiopathic constipation in adults? A review of existing systematic reviews, meta-analyses, and recommendations. Prz Gastroenterol. 2020;15:103-118.

3.2 The Microbiome and Inflammatory Bowel Disease



Kerri L Glassner, DO

Fondren IBD Program, Lynda K and David M Underwood Center for Digestive Disorders, Houston Methodist Hospital and Weill Cornell Medical College, Houston, Texas, USA

Bincy P Abraham, MD, MSc

Fondren IBD Program, Lynda K and David M Underwood Center for Digestive Disorders, Houston Methodist Hospital and Weill Cornell Medical College, Houston, Texas, USA

Eamonn MM Quigley, MD, FRCP, FACP, MACG, FRCPI, MWGO

Lynda K and David M Underwood Center for Digestive Disorders, Houston Methodist Hospital and Weill Cornell Medical College, Houston, Texas, USA

The gut microbiome and inflammation – the basics

The gut microbiome comprises over 100 trillion different microbes, including bacteria, fungi, viruses, and protozoa[1]. In fact, the enteric microbiome comprises 100 times more genes within it than its host[2]. The majority of intestinal bacteria belong to four phyla, Firmicutes, Bacteroidetes, Proteobacteria, and Actinobacteria and, in healthy adults, Firmicutes and Bacteroidetes predominate[3]. Bacterial density increases along the length of the gastrointestinal tract with the colon (the most common site of IBD) containing both the greatest number and diversity of bacteria[4]. The gut microbiome plays a fundamental role in several aspects of host homeostasis: nutrition, immune development, metabolism and defense against pathogens[5].

The microbiome and IBD

Inflammatory bowel disease (IBD) is a chronic immune mediated disease affecting the gastrointestinal tract. The disease is thought to develop as a result of interactions between environmental, microbial, and immune-mediated factors in a genetically susceptible host. Several strands of evidence suggest a role for the microbiome in the pathogenesis of IBD. Data from a number of animal models provides a convincing argument for a fundamental role of an altered microbiome or an aberrant immune response to the microbiome in the development of intestinal inflammation[6]. Thus, a germ-free environment prevents the development of colitis in genetically susceptible mice[7]. In addition, the transfer of pro-inflammatory bacteria or microbiota from diseased mice into healthy mice can induce inflammation[8-9]. Finally, the transfer of naïve CD4+ lymphocytes from healthy mice into mice that lack T and B cells can induce colitis[10-12], and the degree of susceptibility to colitis in these mice is associated with differences in the composition of their gut microbiota[13-14].

In humans, several observations support a role for the microbiome in IBD[15]. For example, disease activity is most evident in areas where bacterial populations are highest and where there is relative stasis of fecal material (the terminal ileum and rectum). Furthermore, fecal diversion has been an effective strategy in the management of Crohn's disease (CD) with remission occurring in the excluded segment of bowel[16-18] only for disease to recur once continuity is restored[19]. In addition, many of the genetic markers associated with IBD are related to engagement of the immune system with microbiota[20-22] and recent studies have demonstrated a role for specific microbes in driving or suppressing inflammation[23]. Also, a variety of interventions that modulate the microbiome, from probiotics to antibiotics and fecal microbiota transplantation/transfer (FMT) have been shown to ameliorate symptoms and inflammation in IBD[24].

1. Genetic mutations, the microbiome and IBD

Many of the genetic mutations that are associated with IBD are related to immune function and, specifically, interactions between the immune system and the microbiome. These genes include NOD2, ATG16L1, CARD9, and CLE-

3.2 The Microbiome and Inflammatory Bowel Disease, continued.

C7A[21-23]. NOD2, nucleotide oligomerization domain 2, encodes an intracellular pattern recognition receptor which interacts with peptidoglycan found in both gram positive and negative bacteria. NOD2 is expressed in intestinal epithelial cells and functions as a defensive factor against intracellular bacteria and contributes to the immune response to commensal microbes [25-27]. Mutations in NOD2 are associated with a decrease in IL-10, an anti-inflammatory cytokine, and increased numbers of mucosa-associated bacteria[28]. NOD2 deficient mice have an altered microbiome with increased susceptibility to colitis compared to wild type mice[29-30]. In CD, NOD2 is associated with ileal disease, an increased risk of post-operative recurrence after ileocecal resection, as well as a more aggressive fistulizing and fibro-stenotic disease phenotype[31-33].

CARD9, caspase recruitment domain-containing protein 9, is a protein located within an adaptor protein caspase recruitment domain involved with DECTIN1(CLEC7A) signaling[24]. DECTIN1 is a pattern recognition receptor which recognizes components of the fungal cell wall[34]. CARD9 signaling occurs in response to the recognition of fungal ligands by DECTIN-1. Alterations in DECTIN1 have been associated with medically refractory UC[35]. CARD9 is also required for inflammatory cytokine production in response to specific bacterial stimuli and viral infection[36]. In humans, inherited CARD9 deficiency has been associated with invasive candida infections of the CNS and digestive tract in previously healthy individuals[37].

2. Environmental risk factors and the microbiome

Environmental factors are known to play a role in the development of IBD; even among identical twins, there is only a 20-50% concordance rate of Crohn's disease[38]. Many of the risk factors that have been identified for IBD are related to the microbiome. These include the hygiene hypothesis, exposure to gastroenteritis, breastfeeding, early antibiotic use, cigarette smoking and diet. The hygiene hypothesis contends that a lack of childhood exposure to a range of microbes may have a negative impact on the development of the adaptive immune response. The change in exposure to microorganisms is attributed to cleaner living, urbanization, and increased antibiotic use. Evidence to support this theory is data suggested by the observation that both the temporal and geographical incidence of IBD seems to parallel the industrialization and urbanization of societies. More recent epidemiological studies suggest that the incidence of IBD has now stabilized in the Western world (United States, Canada, Australia, New Zealand and Western Europe), but continues to increase in South America, Eastern Europe, Asia, and Africa; regions where there has been rapid, recent socioeconomic development[39].

Antibiotic use in the years prior to diagnosis has been associated with the development of IBD and is thought to be related to effects on the commensal microbiota and immune regulation[40-43].

Additional evidence to support a role for the microbiome in IBD includes the effect of infectious gastroenteritis in IBD. Having had an episode of infectious gastroenteritis has been shown in some studies to increase the risk for the subsequent development of IBD by 40%[44].

There is some evidence that breastfeeding is protective against the development of IBD; indeed, human breast milk is microbially diverse and has both probiotic and prebiotic effects[45,46]. Microbiota in breast milk promote immune tolerance, prevent infections and play a role in the maintenance of the epithelial barrier through an immune-mediated influence on intestinal microbiota composition[47-49]. Infants who are breastfed have a lower incidence of gastrointestinal tract infections[50].

Dietary changes, if sufficiently drastic, can alter the intestinal microbiome in as little as 24 hours[51]. Certain diets have been associated with an increased risk for IBD. A population-based case control survey conducted by Bernstein et al found that IBD patients were less likely to have consumed unpasteurized milk or eaten pork[52]. A systematic review by Hou et al found that diets high in total fats, omega-6 fatty acids, and meat were associated with an increased risk of IBD, whereas higher fiber and fruit intakes were associated with a decreased risk for CD, and a high intake of vegetables was associated with a decreased risk for UC[53]. These findings may be explained by dietary induced shifts in the microbiome, such as the decreased abundance of *Firmicutes* with animal-based diets[54].

Cigarette smoking has a complex interaction with IBD being apparently protective against UC but negatively

3.2 The Microbiome and Inflammatory Bowel Disease, continued.

impacting the natural history of CD[55]. Though little studied, there is evidence that gut microbiota of current and former smokers differs from those of non-smokers[56,57].

3. Microbial composition and function in IBD

Numerous studies have described changes in gut microbiota composition related to IBD. These studies have generated much excitement around the diagnostic and prognostic potential of microbiota signatures in IBD. Could such signatures, for example, distinguish between IBD and healthy controls or between CD and UC? Alternately, could microbiota profiling predict risk for future complications, extra-intestinal disease and response to therapy?

When compared to microbiota of healthy individuals, microbiota samples from IBD sufferers demonstrate a decrease in overall diversity and a reduced abundance of anti-inflammatory taxa; with Proteobacteria, and particularly adherent invasive *Escherichia coli*, Pasteurellaceae, Veillonellaceae, *Fusobacterium*, and *Ruminococcus gnavus* being increased and *Clostridium* groups IV and XIVa, *Bacteroides, Suterella, Roseburia, Bifidobacterium,* and *Faecalibacterium prausnitzii* decreased[58].

Transcriptomics studies have emphasized that bacterial functions and not just abundance may also be relevant with a number of studies highlighting differences between the actual functional activity of gut microbiota and their functional potential, as revealed by metagenomics in IBD and, thereby, illustrated the limitations of studies that do not include either metabolomics or metatranscriptomics[59].

Short chain fatty acid producing bacteria are depleted in IBD. Short chain fatty acids including acetate, propionate, and butyrate, are important anti-inflammatory bacterial metabolites and serve as a source of energy for colonic epithelial cells and promote the expansion of regulatory T cells in the colon[60].

Several limitations must be taken into account when considering the previously mentioned alterations of microbial composition and function in IBD. First, the changes seen in mucosal associated microbiota are not always reflected to the same degree in fecal samples. Second, metagenomics reveals functional potential but may not correlate with functional activity. Third, meta-transcriptomics measures actual gene expression; however, there are few studies thus far in IBD. Fourthly, metabolomics measures the actual metabolites produced but the majority of the gut metabolome is uncharacterized. It is also critical to appreciate that alterations in gut microbiota composition in IBD could be the result and not the cause of inflammation in IBD; inflammation resulting in higher oxygen concentrations may create an environment that is toxic to obligate anaerobes and contribute to a diminished mucus layer.

4. Microbiota based strategies in IBD

Several approaches may be taken to the modulation of gut microbiota for therapeutic benefit in IBD. For example, the identification of a deficit in relevant anti-inflammatory bacteria such as *F. prausnitzii* could lead to their augmentation or to the administration of anti-inflammatory molecules, such as the MAM protein, that these bacteria produce. Probiotics, prebiotics and synbiotics have been used in attempts to replenish anti-inflammatory bacteria and their substrates. Conversely, finding inflammatory bacteria that are overexpressed or toxic and using antibiotics or phage therapy to achieve their removal provides yet another approach. Fecal microbiota transfer goes one step further and attempts to reset the entire microbiome. Microbiota could be employed to deliver medications such as genetically modified organisms designed to release anti-inflammatory cytokines or other molecules directly to the site of inflammation. Finally, the microbiome-immune interface has provided multiple targets amenable to therapeutic modulation.

a) Probiotics

While *in vitro* and *in vivo* studies in animal models have shown that probiotics are able to alter the mucosal immune system through engagement with toll-like receptors (TLR's) to promote T-helper 1 cell differentiation, improve intestinal barrier function, increase bacterial diversity, and inhibit the growth of potentially pathogenic bacteria, results, to date, in clinical studies in IBD have not been consistent[61]. There is consistent evidence to support the use of a probiotic cocktail (containing a mixture of four strains of *Lactobacilli*, three strains of *Bifidobacteria*, and *Streptococcus salivarius*) in the primary and secondary (i.e.

3.2 The Microbiome and Inflammatory Bowel Disease, continued.

after induction of remission with antibiotics) prevention of pouchitis[62,63]. There also may be some benefit of probiotics in the induction and maintenance of remission in mild to moderate ulcerative colitis, although outcomes have varied [64,65]. In Crohn's disease, probiotics have not been effective[66].

The application of positive laboratory findings to everyday practice has been hampered by limitations in study design, availability of effective strains and issues related to quality control of available probiotic preparations[61]. Lack of efficacy of probiotics may reflect our failure to identify the ideal strain or combination of strains, a duration of therapy that is too short or timing the intervention too late in the disease course when the inflammatory process is too severe or established to be reversed by a microbial therapy.

b) Antibiotics

Antibiotics play a role in the treatment of IBD in specific scenarios including perianal CD, prevention of post-operative CD, and pouchitis[61,67]. The microbiome plays a central role in driving inflammation of the ileal pouch after colectomy with ileal pouch-anal anastomosis[68]; indeed, pouchitis only occurs after restoration of the fecal stream through the pouch. The antibiotics ciprofloxacin and metronidazole are used as first-line therapy for the treatment of pouchitis, although data supporting their role are quite limited[68].

c) Diet

Studies assessing diet in the treatment of IBD have been notoriously difficult due to the impact of confounding factors and the challenges of achieving long-term patient compliance with dietary changes. In children with Crohn's disease the provision of nutrition exclusively via the enteral route proved to be as effective as corticosteroids; however, long-term adherence is challenging, and disease recurs after a regular diet is resumed[69]. Recently, a randomized controlled trial of the Crohn's disease exclusion diet with partial enteral nutrition in children showed comparable rates of remission to exclusive enteral nutrition[70]. The study showed that after returning to a regular diet, there was a rebound effect on the composition of the microbiome which returned to its baseline composition. There is insufficient evidence to support the use of this strategy in adults[71]. The results of currently ongoing larger randomized controlled trials assessing the use of diet in IBD and their impact on the microbiome are eagerly awaited.

d) Fecal Microbiota Transfer

The evidence supporting FMT in IBD is also rather limited[72]; though there is some evidence to suggest efficacy in UC. Several issues may impact outcomes: IBD phenotype, donor selection, dose and frequency of dosing[73-75]. For example, some trials of FMT in UC showed that specific donor samples produced the majority of the treatment benefit[76,77]. The demonstration that remission in IBD following FMT was associated with the restoration of greater microbial diversity and the engraftment of certain taxa may provide insights into the personalization of microbiota therapy in IBD[78].

Two other scenarios deserve mention but require further studies– the use of FMT to treat *C. difficile* infection in IBD[79] and to treat unresponsive colitis caused by check point inhibitors[80].

Conclusions

The microbiome in IBD is altered compared to that of healthy controls and there is a considerable body of evidence to support a role for the microbiome in disease development and progression. Some, albeit limited, clinical data support the efficacy of treatment strategies that target the microbiome in IBD. Current evidence supports the use of antibiotics to prevent post-operative recurrence in CD, in the treatment of pouchitis, and in perianal disease. Certain probiotics may help in the prevention of pouchitis and, possibly, in the maintenance of remission in mild to moderate UC. Dietary changes can be effective in IBD and, in particular, the use of exclusively enteral nutrition in children with Crohn's disease, but additional studies are needed in adults and in UC. There is limited evidence to support a role for FMT in the treatment of UC but not, as yet, in CD. The microbiome represents a rapidly evolving target which could prove transformative in relation to diagnosing, predicting prognosis and treating IBD.

3.2 The Microbiome and Inflammatory Bowel Disease, continued.

References

1. Nishida A, Inoue R, Inatomi O, et al. Gut microbiota in the pathogenesis of inflammatory bowel disease. Clinical Journal of Gastroenterology 2018;11:1-10.
2. Backhed F. Programming of host metabolism by the gut microbiota. Ann Nutr Metab 2011;58:44-52.
3. Jandhyala SM, Talukdar R, Subramanyam C, et al. Role of the normal gut microbiota. World J Gastroenterol 2015;21:8787-8803.
4. Sartor RB. Microbial influences in inflammatory bowel diseases. Gastroenterology 2008;134:577-94.
5. O'Hara AM, Shanahan F. The gut flora as a forgotten organ. EMBO Rep 2006;7:688-93.
6. Cominelli F, Arseneau K, Rodriguez-Palacios A, et al. Uncovering pathogenic mechanisms of inflammatory bowel disease using mouse models of Crohn's disease-like ileitis: what is the right model? Cell Mol Gastroenterol Hepatol 2017;4:19-32.
7. Veltkamp C, Tonkonogy S, De jong, YP, et al. Continuous stimulation by normal luminal bacteria is essential for the development and perpetuation of colitis in TgÎ26 Mice. Gastroenterology 2001;120:900-913.
8. Schaubeck M, Clavel T, Calasan J, et al. Dysbiotic gut microbiota causes transmissible Crohn's disease-like ileitis independent of failure in antimicrobial defense. Gut 2016;65:225-237.
9. Ohkusa T, Okayasu I, Ogihara T, et al. Induction of experimental ulcerative colitis by Fusobacterium varium isolated from colonic mucosa of patients with ulcerative colitis. Gut 2003;52:79-83.
10. Ostanin D, Bao J, Koboziev I, et al. T cell transfer model of chronic colitis: concepts, considerations, and tricks of the trade. Am J Physiol Gastrointest Liver Physiol 2009;296:G135-G146.
11. Powrie F. T cells in inflammatory bowel disease: protective and pathogenic roles. Immunity 1995;3:171-174.
12. Powrie F, Leach MW, Mauze S, et al. Inhibition of Th1 responses prevents inflammatory bowel disease in scid mice reconstituted with CD45RBhi CD4+ T cells. Immunity 1994;1:553-562.
13. Webb C, Bakker H, Koboziev I, et al. Differential Susceptibility to T cell induced colitis in mice: role of the intestinal microbiota. Inflamm Bowel Dis 2018;24:361-379.
14. Yang I, Eibach D, Kops F, et al. Intestinal microbiota composition of interleukin-10 deficient C57BL/6J mice and susceptibility to Helicobacter hepaticus-induced colitis. PLoS ONE 2013;8:e70783.
15. Somineni HK, Kugathasan S. The microbiome in patients with inflammatory diseases. Clinical Gastroenterology and Hepatology 2019;17:243-255.
16. Harper PH, Lee EC, Kettlewell MG, et al. Role of the faecal stream in the maintenance of Crohn's colitis. Gut 1985;26:279-284.
17. Rutgeerts P, Goboes K, Peeters M, et al. Effect of faecal stream diversion on recurrence of Crohn's disease in the neoterminal ileum. Lancet. 1991;338:771-774.
18. Janowitz HD, Croen EC, Sachar DB. The role of the fecal stream in Crohn's disease: a historical and analytic review. Inflamm Bowel Dis. 1998;4:29-39.
19. D'Haens GR, Geboes K, Peeters M, et al. Early lesions of recurrent Crohn's disease caused by infusion of intestinal contents in excluded ileum. Gastroenterology 1998;114:262-267.
20. Jostins L, Ripke S, Weersma RK, et al. Host-microbe interactions have shaped the genetic architecture of inflammatory bowel disease. Nature 2012;491:119-124.
21. Liu JZ, van Sommeren S, Huang H, et al. Association analyses identify 38 susceptibility loci for inflammatory bowel disease and highlight shared genetic risk across populations. Nat Genet 2015;47:979-986.

3.2 The Microbiome and Inflammatory Bowel Disease, continued.

22. Cohen L, Cho J, Gevers D, et al. Genetic factors and the intestinal microbiome guide development of microbe-based therapies for inflammatory bowel diseases. Gastroenterology 2019;156:2174-2189.
23. Zuo T, Ng SC. The gut microbiota in the pathogenesis and therapeutics of inflammatory bowel disease. Front Microbiol. 2018;9:2247.
24. Khan KJ, Ullman TA, Ford AC, et al. Antibiotic therapy in inflammatory bowel disease: a systematic review and meta-analysis. Am J Gastroenterol. 2011;106:661-673.
25. Hisamatsu T, Suzuki M, Reinecker H, et al. CARD15/NOD2 functions as an antibacterial factor in human intestinal epithelial cells. Gastroenterology 2003;124:993-1000.
26. Kobayashi KS, Chamaillard M, Ogura Y, et al. Nod2-dependent regulation of innate and adaptive immunity in the intestinal tract. Science 2004;307:731-734.
27. Petnicki-Ocwieja T, Hrncir T, Liu TY, et al. Nod2 is required for the regulation of the commensal microbiota in the intestine. Proc Natl Acad Sci USA 2009;106:15813-15818.
28. Swidsinski A, Ladhoff A, Pernthaler A, et al. Mucosal flora in inflammatory bowel disease. Gastroenterology. 2002;122:44-54.
29. Couturier-Maillard A, Secher T, Rehman A, et al. Nod2 mediated dysbiosis predisposes mice ot transmissible colitis and colorectal cancer. J Clin Invest 2013;123:700-711.
30. Al Nabhani Z, Lepage P, Mauny P, et al. NOD2 deficiency leads to a specific and transmissible mucosa-associated microbial dysbiosis which is independent of the mucosa barrier defect. J Crohns Colitis 2016;10:1428-1436.
31. Abreu M, Taylor K, Ying-Chao L, et al. Mutations in NOD2 are associated with fibrostenosing disease in patients with Crohn's disease. Gastroenterology 2002;123:679-688.
32. Helio T, Halme L, Lappalainen M, et al. CARD15/NOD2 gene variants are associated with familially occurring and complicated forms of Crohn's disease. Gut 2003;52:558-562.
33. Alvarez-lobos M, Arostegui J, Sans M, et al. Crohn's disease patients carrying Nod2/CARD15 gene variants have an increased and early need for first surgery due to stricturing disease and higher rate of surgical recurrence. Ann Surg 2005;242:693-700.
34. Drummond RA, Franco LM, Lionakis MS. Human CARD9: a critical molecule of fungal immune surveillance. Front Immunol 2018;9:1836.
35. Iliev ID, Funari VA, Taylor KD, et al. Interactions between commensal fungi and the C-type lectin receptor Dectin-1 influence colitis. Science 2012;336(6086):1314-7.
36. Lanternier F, Mahdaviani SA, Barbati E, et al. Inherited CARD9 deficiency in otherwise healthy children and adults with Candida species-induced meningoencephalitis, colitis, or both. J Allergy Clin Immunol 2015;135:1558-68.
37. Sokol H, Conway KL, Zhang M, et al. Card9 mediates intestinal epithelial cell restitution, T-helper 17 responses, and control of bacterial infection in mice. Gastroenterology 2013;145:591-601.
38. Halfvarson J, Bodin L, Tysk, et al. Inflammatory bowel disease in a Swedish twin cohort: a long-term follow-up of concordance and clinical characteristics. Gastroenterology 2003;124:1767-1773.
39. Ng S, Shi H, Hamidi N, et al. Worldwide incidence and prevalence of inflammatory bowel disease in the 21st century: a systematic review of population-based studies. Lancet 2017;390:2769-78.
40. Shaw SY, Blanchard JF, Bernstein CN. Association between the use of antibiotics in the first year of life and pediatric inflammatory bowel disease. Am J Gastroenterol 2010;105:2687-92.

3.2 The Microbiome and Inflammatory Bowel Disease, continued.

41. Shaw SY, Blanchard JF, Bernstein CM. Association between early childhood otitis media and pediatric inflammatory bowel disease: an exploratory population-based analysis. J Pediatrics 2013;162:510-514.
42. Shaw SY, Blanchard JF, Bernstein CM. Association between the use of antibiotics and new diagnoses of Crohn's disease and ulcerative colitis. Am J Gastroenterol 2011;106:2133-42.
43. Ungaro R, Bernstein CN, Gearry R, et al. Antibiotics associated with increased risk of new-onset Crohn's disease but not ulcerative colitis: a meta-analysis. Am J Gastroenterol 2014;109):1728-38.
44. Porter CK, Tribble DR, Aliaga PA, et al. Infectious gastroenteritis and risk of developing inflammatory bowel disease. Gastroenterology 2008;135:781-786.
45. Moossavi S, Miliku K, Sepehri S, et al. The prebiotic and probiotic properties of human milk: implications for infant immune development and pediatric asthma. Front Pediatr. 2018;6:197.
46. Parigi SM, Eldh M, Larssen P, et al. Breast milk and solid food shaping intestinal immunity. Front Immunol 2015;6:415.
47. Rogier EW, Frantz AL, Bruno ME, et al. Lessons from mother: long term impact of antibodies in breast milk on the gut microbiota and intestinal immune system of breastfed offspring. Gut Microbes 2014;5:663-668.
48. Coppa GV, Zampini L, Galeazzi T, et al. Human milk oligosaccharides inhibit the adhesion to Caco-2 cells of diarrheal pathogens: Escherichi coli, Vibrio cholerae, and Salmonella fyris. Pediatric Research 2006;59:377-382.
49. Duijts L, Jaddoe V, Hofman A, et al. Prolonged and exclusive breastfeeding reduces the risk of infectious diseases in infancy. Pediatrics 2010;126:e18-e25.
50. Azad MB, Konya T, Maughan H, et al. Gut microbiota of healthy Canadian infants: profiles by mode of delivery and infant diet at 4 months. CMAJ 2013;185:385-394.
51. David L, Maurice C, Carmody R, et al. Diet rapidly and reproducibly alters the human gut microbiome. Nature 2014;505:559-63.
52. Bernstein CN, Rawsthorne P, Cheang M, et al. A population-based case control study of potential risk factors for IBD. Am J Gastroenterol 2006;101:993-1002.
53. Hou JK, Abraham BP, El-Serag H. Dietary intake and risk of developing inflammatory bowel disease: a systematic review of the literature. Am J Gastroenterol 2011;106:563-73.
54. Quevrain E, Maubert MA, Michon C, et al. Identification of an anti-inflammatory protein from Faecalibacterium prausnitzii, a commensal bacterium deficient in Crohn's disease. Gut 2016;65:415-425.
55. Kuenzig ME, Lee SM, Eksteen B, et al. Smoking influences the need for surgery in patients with the inflammatory bowel diseases: a systematic review and meta-analysis incorporating disease duration. BMC Gastroenterol. 2016;16:143.
56. Lee SH, Yun Y, Kim SJ, et al. Association between Cigarette Smoking Status and Composition of Gut Microbiota: Population-Based Cross-Sectional Study. J Clin Med. 2018;7: E282.
57. Shanahan ER, Shah A, Koloski N, et al. Influence of cigarette smoking on the human duodenal mucosa-associated microbiota. Microbiome. 2018;6:150.
58. Sartor RB, Wu GD. Roles for intestinal bacteria, viruses, and fungi in pathogenesis of inflammatory bowel diseases and therapeutic approaches. Gastroenterology 2017;152:327-39.
59. Kostic AD, Xavier RJ, Gevers D. The microbiome in inflammatory bowel disease: current status and the future ahead. Gastroenterology 2014;146:1489-99.
60. Venegas D, De la Fuente M, Landskron G, et al. Short chain fatty acids (SCFAs)-mediated gut epithelial and immune regulation and its relevance for inflammatory bowel diseases. Front Immunol 2019;10:277.
61. Abraham BP, Quigley EMM. Probiotics in inflammatory bowel disease. Gastroenterology Clin North Am 2017;46:769-782.

3.2 The Microbiome and Inflammatory Bowel Disease, continued.

62. Gionchetti P, Rizzello F, Helwig U, et al. Prophylaxis of pouchitis onset with probiotic therapy: a double-blind, placebo-controlled trial. Gastroenterology 2003;124:1202-1209.
63. Mimura T, Rizzello F, Helwig U, et al. Once daily high dose probiotic therapy (VSL#3) for maintaining remission in recurrent or refractory pouchitis. Gut 2004;53:108-114.
64. Iheozor-Ejiofor Z, Kaur L, Gordon M, et al. Probiotics for maintenance of remission in ulcerative colitis. Cochrane Database Syst Rev. 2020;3:CD007443.
65. Kaur L, Gordon M, Baines PA, et al. Probiotics for induction of remission in ulcerative colitis. Cochrane Database Syst Rev. 2020;3CD005573.
66. Limketkai BN, Akobeng AK, Gordon M, Adepoju AA. Probiotics for induction of remission in Crohn's disease. Cochrane Database Syst Rev. 2020 Jul 17;7:CD006634.
67. Lichtenstein G, Loftus E, Isaacs K, et al. ACG clinical guideline: management of Crohn's disease in adults. Am J Gastroenterol 2018;113:481-517.
68. Batista D, Raffals L. Role of intestinal bacteria in the pathogenesis of pouchitis. Inflamm Bowel Dis 2014;20:1481-1486.
69. Dziechciarz P, Horvath A, Shamir R, et al. Meta-analysis: enteral nutrition in active Crohn's disease in children. Aliment Pharmacol Ther 2007;26:795-806.
70. Levine A, Wine E, Assa A, et al. Crohn's disease exclusion diet plus partial enteral nutrition induces sustained remission in a randomized controlled trial. Gastroenterology 2019;157:440-450.
71. Limketkai BN, Iheozor-Ejiofor Z, Gjuladin-Hellon T, et al. Dietary interventions for induction and maintenance of remission in inflammatory bowel disease. Cochrane Database Syst Rev. 2019;2:CD012839.
72. Imdad A, Nicholson MR, Tanner-Smith EE, et al. Fecal transplantation for treatment of inflammatory bowel disease. Cochrane Database Syst Rev. 2018;11:CD012774.
73. Yalchin M, Segal JP, Mullish BH, et al. Gaps in knowledge and future directions for the use of faecal microbiota transplant in the treatment of inflammatory bowel disease.Therap Adv Gastroenterol. 2019 Nov 25;12:1756284819891038.
74. Lai CY, Sung J, Cheng F, et al. Systematic review with meta-analysis: review of donor features, procedures and outcomes in 168 clinical studies of faecal microbiota transplantation. Aliment Pharmacol Ther. 2019;49:354-363.
75. Levy A, Allegretti J. Insights into the role of fecal microbiota transplantation for the treatment of inflammatory bowel disease. Ther Adv Gastroenterol 2019.12:1-10.
76. Paramsothy S, Kamm MA, Kaakoush NO, et al. Multidonor intensive faecal microbiota transplantation for active ulcerative colitis: a randomized placebo-controlled trial. Lancet 2017;389:1218-1228.
77. Moayyedi P, Surette MG, Kim PT, et al. Fecal microbiota transplantation induces remission in patients with active ulcerative colitis in a randomized controlled trial. Gastroenterol 2015;149:102-109.
78. Paramsothy S, Nielsen S, Kamm MA, et al. Specific bacteria and metabolites associated with response to fecal microbiota transplantation in patients with ulcerative colitis. Gastroenterology 2019;156:1440-1454.
79. Tariq R, Disbrow MB, Dibaise JK, et al. Efficacy of Fecal Microbiota Transplantation for Recurrent C. Difficile Infection in Inflammatory Bowel Disease. Inflamm Bowel Dis. 2020;26:1415-1420.
80. Pezo RC, Wong M, Martin A. Impact of the gut microbiota on immune checkpoint inhibitor-associated toxicities.Therap Adv Gastroenterol. 2019;12:1756284819870911.

3.3 Small Intestinal Bacterial Overgrowth

Maher Malaeb, MD

Division of Gastroenterology, American University of Beirut Medical Center
Beirut, Lebanon

Ala I. Sharara, MD

Division of Gastroenterology, American University of Beirut Medical Center
Beirut, Lebanon

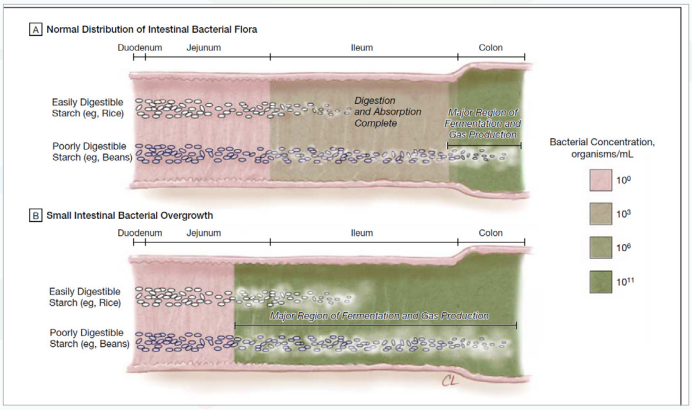


FIGURE. Distribution of intestinal bacterial flora in (A) normal gut and (B) small intestinal bacterial overgrowth (SIBO). Fermentation of both easily digestible and poorly digestible starches is increased in SIBO (reprinted with permission from Lin HC. JAMA 2004;292:852-8.)

Introduction

Small intestinal bacterial overgrowth (SIBO) is a condition characterized by increased number and/or change in the type of bacteria in the small bowel [1]. The microbial environment in the proximal small intestine is less diverse and has a lower but more dynamic biomass given the rapidly altering luminal conditions. Multiple factors are involved including short transit time, intermittent food delivery and the influx of bile and digestive enzymes [2]. The bacterial populations increase from the proximal to the distal segments of the small intestine and colon with the duodenum containing approximately 10^{4-5} colony-forming units (CFU)/mL reaching 10^{7-8} CFU/mL in the distal ileum (Figure) [2]. The intestinal microbiome is complex. The proportion of gram-positive to gram-negative bacteria increases from proximal to distal segments; similarly facultative anaerobic to strict anaerobic species [2]. SIBO may occur due to not only an increase in the number of small bowel bacteria but also when there is an alteration in the gut microbiota such that the microbes that are usually present in the distal gut shift more proximally [3].

Etiology

There are multiple factors that act as endogenous defense in preventing bacterial overgrowth including an intact ileocecal valve, the acidic milieu of the stomach, intestinal motility, presence of immunoglobulins in intestinal secretions, and the bacteriostatic nature of the biliary and pancreatic secretions [4, 5].

The etiology of SIBO is usually complex and multifactorial including:

- Disorders of protective antibacterial mechanisms (e.g. achlorhydria [4], pancreatic exocrine insufficiency [6], immunodeficiency syndromes [5])
- Anatomical abnormalities [1] (e.g. small intestinal obstruction, diverticula, fistulae, surgical blind loop, previous ileocecal resections)
- Motility disorders (e.g. systemic sclerosis [7], diabetic enteropathy [8], post-radiation enteropathy [1], liver cirrhosis [9])

SIBO may mask or exacerbate the symptoms of some diseases such as celiac disease and irritable bowel disease. It is more common in some extra-intestinal disorders such as scleroderma and obesity [10].

3.3 Small Intestinal Bacterial Overgrowth, continued.

Clinical Presentation and Diagnosis

The classic presentation include steatorrhea, abdominal bloating, and weight loss. The most common clinical manifestations of SIBO are watery diarrhea, bloating, abdominal pain and distension in addition to deficiency of multivitamins (B12, D, A, and E) and iron [11]. Vitamin K and folate levels are usually not affected because of bacterial synthesis [3, 11]. Although there is no gold standard diagnostic test, small bowel culture is widely accepted as the "best diagnostic method" for establishing a diagnosis of SIBO [12]. The test of choice is jejunal aspiration and culture. However, in practice, most aspirates are obtained from the duodenum during upper endoscopy [11]. A threshold of $\geq 10^3$ CFU/mL is considered positive when performing duodenal aspirate and culture, because of very low bacterial counts in this more acidic environment. However, some have suggested a higher threshold of $\geq 10^5$ CFU/mL based on traditional microbiological standards of jejunal aspirate culture [12]. The limitations of small bowel culture include cost, its invasive nature, potential inability to detect bacterial strains that are difficult to grow under standard culture conditions, detection of proximal SIBO only due to the inability of the scope to reach the distal small bowel, leading to false-negatives results. Furthermore, sample contamination is possible including technical limitations, such as esophageal and oral bacterial contamination, leading to false-positives results [11, 12].

Breath testing is an alternative noninvasive method to diagnose SIBO. However, there is currently no standard methodology for breath testing. During breath testing, the patient is asked to ingest a carbohydrate substrate, which is metabolized into hydrogen and methane when exposed to luminal microbes. Some of these gases are absorbed into the blood stream from the GI tract and then exhaled into the lungs. Thus analysis of breath samples provides an indirect measure of SIBO. Glucose and lactulose are commonly used as breath test substrates for detecting SIBO [12].

The North American Consensus provided recommendations for preparing patients for breath testing [13]:

1. Antibiotics should be avoided for 4 weeks prior to the breath test.
2. Promotility drugs and laxatives should be stopped ≥ one week prior to testing.
3. Fermentable foods such as complex carbohydrates should be avoided on the day prior to breath testing.
4. The fasting period prior to testing should be 8–12 h.
5. Smoking should be avoided on the day of testing.
6. Physical activity should be limited during breath testing.
7. It is not necessary to stop proton pump inhibitors prior to breath testing.

An absolute increase in hydrogen by ≥20 ppm above baseline within 90 minutes on the lactulose/glucose breath test is diagnostic of SIBO. In addition to small intestine culture and breath testing, novel capsule technologies that can sample small bowel bacteria is also emerging. These technologies could provide a more direct, non-invasive and accurate evaluation for SIBO; however, further clinical trials and validation are needed [12].

Treatment

The goal of treatment for patients with SIBO is symptom relief, which is typically achieved by treatment with antibiotics. A variety of antibiotics have been used, the most common of which include ciprofloxacin, metronidazole, neomycin, rifaximin, and tetracycline [11]. Rifaximin, a non-systemic antibiotic, is currently the most studied agent for patients with SIBO. Multiple studies have demonstrated its efficacy as well as safety and minimal impact on bacterial resistance [12]. However, some patients may remain symptomatic after antibiotic therapy, suggesting that the bacteria may be resistant to antibiotics and/or other underlying conditions may potentially be the cause of symptoms such as dysmotility or PPI use [12]. Nutritional support is an important component in treating patients with SIBO especially those with weight loss or vitamin or mineral deficiencies. Supplementation and maintenance of vitamin B_{12} and fat-soluble vitamins along with correction of calcium and magnesium deficiencies are key components in the management [14].

Several non-pharmacologic treatments have been proposed because of the cost and potential adverse effects

3.3 Small Intestinal Bacterial Overgrowth, continued.

of antibiotics. Elemental diet is an example as it contains predigested micronutrients that are mostly absorbed within the proximal intestines and thus limit the delivery of nutrients to bacteria in the distal portion of the small bowels. This type of diet has been shown to lead to breath test normalization and improvement in symptoms. However, it is generally not palatable and difficult to maintain adherence and compliance by the patient [12].

Suggested References

1. Bures, J., et al., *Small intestinal bacterial overgrowth syndrome.* World J Gastroenterol, 2010. **16**(24): p. 2978-90.
2. Kastl, A.J., Jr., et al., *The Structure and Function of the Human Small Intestinal Microbiota: Current Understanding and Future Directions.* Cell Mol Gastroenterol Hepatol, 2020. **9**(1): p. 33-45.
3. Adike, A. and J.K. DiBaise, *Small Intestinal Bacterial Overgrowth: Nutritional Implications, Diagnosis, and Management.* Gastroenterol Clin North Am, 2018. **47**(1): p. 193-208.
4. Lewis, S.J., et al., *Altered bowel function and duodenal bacterial overgrowth in patients treated with omeprazole.* Aliment Pharmacol Ther, 1996. **10**(4): p. 557-61.
5. Jones, R.M. and A.S. Neish, *Recognition of bacterial pathogens and mucosal immunity.* Cell Microbiol, 2011. **13**(5): p. 670-6.
6. Batt, R.M., *Exocrine pancreatic insufficiency.* Vet Clin North Am Small Anim Pract, 1993. **23**(3): p. 595-608.
7. Sakkas, L.I., et al., *Intestinal Involvement in Systemic Sclerosis: A Clinical Review.* Dig Dis Sci, 2018. **63**(4): p. 834-844.
8. Gotfried, J., S. Priest, and R. Schey, *Diabetes and the Small Intestine.* Curr Treat Options Gastroenterol, 2017. **15**(4): p. 490-507.
9. Ghosh, G. and A.B. Jesudian, *Small Intestinal Bacterial Overgrowth in Patients With Cirrhosis.* J Clin Exp Hepatol, 2019. **9**(2): p. 257-267.
10. Losurdo, G., et al., *The Influence of Small Intestinal Bacterial Overgrowth in Digestive and Extra-Intestinal Disorders.* Int J Mol Sci, 2020. **21**(10).
11. Krajicek, E.J. and S.L. Hansel, *Small Intestinal Bacterial Overgrowth: A Primary Care Review.* Mayo Clin Proc, 2016. **91**(12): p. 1828-1833.
12. Rao, S.S. and J. Bhagatwala, *Small intestinal bacterial overgrowth: Clinical features and therapeutic management.* Clinical and translational gastroenterology, 2019. **10**(10).
13. Rezaie, A., et al., *Hydrogen and Methane-Based Breath Testing in Gastrointestinal Disorders: The North American Consensus.* Am J Gastroenterol, 2017. **112**(5): p. 775-784.
14. Dukowicz, A.C., B.E. Lacy, and G.M. Levine, *Small intestinal bacterial overgrowth: a comprehensive review.* Gastroenterol Hepatol (N Y), 2007. **3**(2): p. 112-22.

3.4 Microbiome and Liver Disease

Leonid Lazebnik, MD, PhD

President
Gastroenterological Scientific Society of Russia
Department of Outpatient Therapy
A.I. Yevdokimov Moscow State University of Medicine and Dentistry
Moscow, Russia

Stanislav Sitkin, MD, PhD

Department of Internal Diseases, Gastroenterology and Dietetics
North-Western State Medical University named after I.I. Mechnikov
Institute of Perinatology and Pediatrics
Almazov National Medical Research Centre
St. Petersburg, Russia

Worldwide burden of liver disease

The incidence of chronic liver disease (CLD), and especially non-alcoholic fatty liver disease (NAFLD), is growing steadily, becoming a worldwide burden, not only medical but also financial. The global prevalence of NAFLD, the most common liver disease, is about 25% [1]. Since the proportion of NAFLD among CLD is estimated at about 60–75%, the total number of patients with CLD in the world may be in the range of 2.5–3 billion people. The three main causes of mortality in CLD, rising exponentially, are liver cirrhosis, hepatocellular carcinoma (HCC), and chronic viral hepatitis. Cirrhosis and HCC cause 3.5% of all deaths worldwide [1]. The leading contributor to mortality is cirrhosis, with more than 1.32 million deaths (2017), which is about 1.5 times the number of deaths in 1990 [2]. Liver cancer, including HCC, was responsible for 781,631 deaths in 2018 [3]. Mortality from chronic viral hepatitis is estimated at more than 1.2 million deaths per year [4], including deaths from viral hepatitis-related cirrhosis and carcinoma.

Gut microbiome, dysbiosis and gut-liver axis in liver disease

Dysbiotic changes in the gut microbiota occur in most liver diseases, suggesting both its contribution to CLD pathogenesis and the adverse effect of the disease on the microbiome. The most significant changes in the microbiota were found for such liver diseases as NAFLD, alcohol-related liver disease (ARLD), liver cirrhosis, hepatic encephalopathy (HE), autoimmune liver disease, especially primary sclerosing cholangitis (PSC), HCC, chronic viral hepatitis caused by HBV/HCV infection, and intestinal failure-associated liver disease [5, 6, 7]. Microbiota changes are usually disease-specific, however, there are some features common to CLD-associated dysbiosis, such as reduced bacterial diversity, the presence of endotoxemia, a relative decrease of beneficial autochthonous bacteria, such as Lachnospiraceae, as well as a relative overgrowth of opportunistic pathogens and pathobionts, such as Proteobacteria, Enterobacteriaceae, Staphylococcaceae, Enterococcaceae, Porphyromonadaceae, *Escherichia*, *Streptococcus* [8].

Along with taxonomic changes in the gut microbiota, patients with CLD develop so-called functional (metabolic) dysbiosis. Changes in microbial metabolism may be of greater importance in the pathogenesis of CLD than changes in the composition of the microbiota [9]. The hypothesis that intestinal dysbiosis is caused not so much by compositional changes in the microbiome as by disorders of its metabolism and the metabolome is a greater predictor of dysbiosis than the taxonomic composition of the microbiota, is discussed and increasingly confirmed [10].

The metabolic potential of bacterial genes is so high that it suggests that the microbiome is a "hidden metabolic organ" [11]. The gut microbiota can produce many bioactive metabolites through the transformation and degradation of food and host-derived substances or by bacterial cross-feeding. These metabolites play a key role in the host-microbiota interactions, and naturally, an imbalance of the gut microbiome and related metabolites (both taxonomical and functional dysbiosis) contributes to the onset and progression of CLD [12, 13].

The intestinal microbiome, liver, immune system, and brain communicate extensively through the intestinal barrier,

3.4 Microbiome and Liver Disease, continued.

biliary tract, portal vein and systemic mediators (large and small molecules) of food, bacterial and endogenous origin [14].

The main microbiota-related molecules and metabolites of interest in CLD are lipopolysaccharide (LPS) known as endotoxin, short-chain fatty acids (SCFA), bile acids (BAs), choline (trimethylamine [TMA], trimethylamine-N-oxide [TMAO]) and tryptophan (indoles, including indole-3-acetic acid, indole-3-propionic acid [IPA], indole-3-lactic acid, indole-3-carboxylic acid, and tryptamine) metabolites, branched-chain amino acids (BCAA), ethanol, succinate, phenylacetic acid and 3-(4-hydroxyphenyl) lactate, and some other metabolites [12, 15].

Gut dysbiosis can contribute to the development and progression of CLD towards cirrhosis via endotoxemia, intestinal barrier dysfunction, and BA changes [16]. Dysbiosis-related intestinal barrier changes lead to translocation of bacteria and pathogen-associated molecular patterns (PAMPs). PAMPs such as LPS, bacterial and viral RNAs activate TLR4 on Kupffer and other liver immune cells to induce hepatic inflammation that contributes to CLD [17]. In addition to gut dysbiosis, microbiota-mediated inflammation of the intestinal mucosa and mucosal immune system damage can play an important role in the pathogenesis of CLD such as NAFLD [18].

The other most common patterns of microbiota changes in liver disease are that dysbiosis usually correlates with endotoxemia, appears early in NAFLD and ARLD, and progressively worsens with increasing CLD severity. Microbial metabolites, such as BAs, choline metabolites (TMA), ethanol, phenylacetic acid, 3-(4-hydroxyphenyl) lactate, and a few others, are the main proponents of dysbiosis, and changes in its profile can affect CLD progression. At the onset of liver cirrhosis, taxonomical and functional dysbiosis deteriorate and contribute to complications such as HE and acute-on-chronic liver failure and may be a predictor of readmission and death [12, 15, 16].

Non-alcoholic fatty liver disease and gut microbiome

NAFLD is associated with obesity, insulin resistance, type 2 diabetes mellitus, arterial hypertension, dyslipidemia, and metabolic syndrome. A subtype of NAFLD classified as non-alcoholic steatohepatitis (NASH) progresses to fibrosis and cirrhosis, HCC, and liver transplantation. Complications of NASH are becoming a health and economic burden for patients and society. NASH is an important cause of liver cirrhosis, outstripping viral hepatitis in terms of cirrhosis growth rate. From 1990 to 2017, the prevalence of compensated cirrhosis due to NASH more than doubled and more than tripled for decompensated cirrhosis due to NASH [2].

Until recently, the “two hits” theory in the NAFLD pathogenesis was applied, where the first hit is the steatosis development, and the second hit is steatohepatitis. The outdated concept of “two hits” has now been replaced by the “multiple hits” hypothesis, which more accurately reflects the complex mechanisms that trigger the onset and progression of the NAFLD. This concept includes a number of pathogenetic factors such as insulin resistance, adipose tissue hormones, overweight/obesity, diet, genetic and epigenetic factors, as well as the gut-liver axis, which appears to play a key role in the development and progression of NAFLD. The leading players on this axis are the gut microbiota, bacterial metabolites, and intestinal barrier [19].

Initially, it was shown that microbiota can modulate BA metabolism and their de novo synthesis in the liver through a feedback mechanism. BAs are powerful signaling molecules that can affect insulin sensitivity and fat metabolism in the liver, which play an important role in the NAFLD pathogenesis [20].

Intestinal dysbiosis increases intestinal permeability and metabolic endotoxemia, followed by steatosis, steatohepatitis, and liver fibrosis against the background of TLR-4 activation and increased TNF α, IL-1β, IL-6 secretion [21, 22]. One of the causes of NAFLD is a deficiency of choline, an important component of cell and mitochondrial membranes. It was shown that choline deficiency can be caused not only by its lack in the food, but also by a high level of choline-utilizing bacteria, mainly represented by Enterobacteriaceae, and especially by the genus *Escherichia* [23]. In addition, gut bacteria produce enzymes that catalyze the conversion of choline to toxic methylamines (dimethylamine and TMA). The metabolism of these amines

3.4 Microbiome and Liver Disease, continued.

in the liver and their transformation into TMAO can contribute to the liver inflammation [24]. Differences in fecal levels of Gammaproteobacteria and Erysipelotrichia are directly associated with NAFLD associated with choline deficiency [25].

Key microbial factors associated with the NAFLD pathogenesis within the framework of the "multiple hits" hypothesis are [21, 26, 27, 28] (1) increased intestinal permeability ("leaky gut" concept); (2) translocation of bacteria and LPS across the damaged intestinal barrier with subsequent activation of TLR-4, pro-inflammatory molecules and cytokines secretion (TNF α, IL-1β, IL-6, etc.) and low-grade liver inflammation; (3) increased ethanol production by dysbalanced gut microbiota (it is possible that the increased level of ethanol may be associated with insulin-dependent disturbances of liver alcohol dehydrogenase activity); (4) choline metabolism alterations; (5) BA metabolism disturbance leading to alterations in farnesoid X receptor (FXR) signaling; (6) changes in bacterial production and intestinal absorption of SCFA; (7) microbiota-related disruptions in amino acid homeostasis.

Dysbiotic changes in NAFLD are variable, but there are several common patterns: an increase in the proportion of Bacteroidetes and the level of *Bacteroides* spp., a decrease in Firmicutes, a relative increase in Proteobacteria, Enterobacteriaceae, and especially the genus *Escherichia* [29]. More severe fibrosis (F≥2) was also associated with the predominance of *Escherichia*/*Shigella* and Enterobacteriaceae [30]. Along with an increase in *Bacteroides* and *Escherichia*, in patients with NASH, the levels of *Prevotella*, *Faecalibacterium*, *Anaerosporobacter*, and *Oscillospira* decrease [22]. In patients with NAFLD and severe fibrosis/cirrhosis (F3/F4), there was a significant decrease in the proportion of gram-positive Firmicutes and an increase in the proportion of gram-negative Proteobacteria (including *Escherichia coli*) [31]. An increase in gram-negative Porphyromonadaceae and Bacterioidaceae (both Bacteroidetes) in NASH patients with cirrhosis was previously identified in an American study [32]. The levels of other gram-negative bacteria such as Succinivibrionaceae (Proteobacteria), *Parabacteroides* (Bacteroidetes), and *Allisonella* (class Negativicutes, Firmicutes) can also increase in NAFLD [33]. Several studies have shown a decrease in butyrate-producing bacteria such as *Faecalibacterium prausnitzii* and *Eubacterium rectale* in patients with NASH and severe fibrosis [31, 33].

For the majority of adult cases of NAFLD, it can be assumed that there is a specific NAFLD-associated intestinal dysbiosis characterized by a distinct shift towards endotoxin-producing gram-negative bacteria, primarily the Enterobacteriaceae family and the related genus *Escherichia* (Proteobacteria), as well as the genus *Bacteroides* (Bacteroidetes) [22, 34]. However, not all gram-negative bacteria show an increase in NAFLD. For example, the level of *Prevotella* (Bacteroidetes), on the contrary, decreases in patients with NAFLD, indicating the potential protective effect of these microorganisms [18, 22, 30, 35]. In turn, some gram-positive bacteria can contribute to the progression of the disease. For example, a Franco-American study showed a positive relationship between gram-positive bacteria of the genus *Ruminococcus* and severe fibrosis (F≥2) in NAFLD [35].

An imbalance in the gut microbiota, characterized by an increase in some gram-negative taxa (*Escherichia*), is likely to be observed already in the early stages of NAFLD, while the progression of the disease is associated with more complex changes in the microbial composition. It is possible that different stages of NAFLD (from steatosis to cirrhosis) are accompanied by specific changes in the microbiota (from steatosis-associated dysbiosis to cirrhosis-associated dysbiosis, respectively), which require clarification, however, the general trend towards an increase in gram-negative bacteria persists both in severe fibrosis and in liver cirrhosis [30, 31, 32].

In addition to dysbiosis, SIBO is detected in 40–70% of patients with NAFLD, which also plays a role in the pathogenesis of this disease, contributing to increased intestinal permeability and endotoxemia and promoting both steatosis and inflammation [5]. It is significant that in patients with SIBO, the incidence of NAFLD was 2.6 times higher than in SIBO-negative individuals [36].

The role of *Helicobacter pylori* infection in the pathogenesis of CLD, including NAFLD, chronic viral hepatitis, and HCC is discussed. However, the available data are highly contradictory [37]. Recent research suggests that *H. pylori*

3.4 Microbiome and Liver Disease, continued.

infection may be an independent risk factor for NAFLD, which may increase the severity of NAFLD via promoting liver function damage, glycometabolism, lipid metabolism, inflammatory response, and metabolic syndrome [38]. The mechanisms involving *Helicobacter* species in NAFLD pathogenesis are to be studied in the future and may be just part of the "multiple hits" theory [39].

Alcohol-related liver disease

Over 50% of deaths related to cirrhosis worldwide are attributed to alcohol consumption [1]. Gut microbiota contributes to the ARLD via different mechanisms. Intestinal dysbiosis in patients with alcoholic hepatitis lead to increased gut permeability and bacterial and LPS translocation, immune system disturbances, increase in hepatic inflammation and changes in microbial metabolism, especially in BA biotransformation [40, 41].

In patients with substantial liver fibrosis, gut microbiota dysbiosis occurs in parallel to liver injury and is characterized by an increase in endotoxin-producing bacteria and a reduction in autochthonous taxa [40]. Functional dysbiosis differs between ARLD stages and conditions, with the most pronounced differences between drinking patients with cirrhosis and those with alcoholic hepatitis.

Patients with alcoholic liver cirrhosis (ALC) had a significantly higher abundance of Enterobacteriaceae and Halomonadaceae, lower Lachnospiraceae, Ruminococcaceae, and Clostridialies XIV, high endotoxin and a lower ratio of "good vs. bad" taxa abundance (termed the Cirrhosis Dysbiosis Ratio [CDR]) [32]. A recent Russian study found that gut dysbiosis in ALC is more pronounced than in alcoholic dependence syndrome (ADS), and is characterized by a depletion of commensal Bacteroidales and a rise of some oral taxa such as *Lactobacillus salivarius*, *Veillonella parvula*, and *Streptococcus salivarius*. This effect could be mediated by abnormal BA metabolism [42]. A common feature in both ALC and ADS patients was the dominance of *Bifidobacterium* and *Lactobacillus*, suggesting a reconsideration of the use of probiotic products based on the species from these two genera. One of the causes for the enrichment of these genera could be their involvement in the gut-brain axis. Microbial functions enriched in ALC were glutathione and porphyrin metabolism, and biosynthesis of siderophore group nonribosomal peptides, and some other [42]. ALRD also affects the gut-brain axis, which could hasten the onset of HE and its progression [40].

Liver cirrhosis

Cirrhosis is accompanied by progressive changes in the gut microbiota, which become more severe with decompensation. Gut microbial composition in patients with cirrhosis characterized by the relative decrease of beneficial autochthonous taxa, such as Lachnospiraceae, Ruminococcaceae, and Clostridiales XIV, and overgrowth of potential pathogens and pathobionts, such as Proteobacteria, Fusobacteria, Enterobacteriaceae, Bacteroidaceae, Staphylococcaeae, Streptococcaceae, and Enterococcaceae. Dysbiosis is associated with disease progression and endotoxemia [32, 43]. A reduction in beneficial autochthonous bacteria producing SCFAs, anti-inflammatory molecules, and antimicrobial peptides leads to energy deficiency in the intestinal epithelium, inflammation, and intestinal barrier disruption in cirrhosis, like inflammatory bowel disease (IBD) [44]. The growth of Enterobacteriaceae and increased intestinal permeability lead to endotoxemia, which aggravates and complicates cirrhosis [5]. The proportion of phylum Bacteroidetes was significantly reduced, whereas Proteobacteria and Fusobacteria were highly enriched in the cirrhosis group.

Dysbiosis increases with worsening cirrhosis, accompanied by HE and infection, and is characterized by a lower CDR and higher relative abundance of gram-negative bacteria (Enterobacteriaceae, Bacteroidaceae) [32]. CDR was proposed as a useful quantitative index to describe microbiota changes in cirrhosis progression. CDR is the ratio of potentially beneficial bacteria (Lachnospiraceae + Ruminococcaceae + *Clostridium* Cluster XIV + Veillonellaceae) in the numerator and potentially pathogenic taxa (Enterobacteriaceae + Bacteroidaceae) as the denominator. CDR worsened with the development of the first episode of HE, and was worse in patients with decompensated cirrhosis, and in those who were subsequently hospitalized [32, 45].

Quantitative metagenomics has revealed significant differences between patients with cirrhosis and healthy individuals in more than 75,000 microbial genes. Interestingly, most taxa (>50%) enriched in cirrhotic patients

3.4 Microbiome and Liver Disease, continued.

were of oral origin, suggesting a massive invasion of the gut by oral bacterial species in liver cirrhosis [46]. Some invasive species were significantly correlated with the disease severity, suggesting their involvement in cirrhosis pathogenesis. The Patient Discrimination Index (PDI), based on a combination of 15 microbial genes, highly specifically discriminated patients from healthy controls, confirming its potential use for identifying and monitoring patients with liver cirrhosis [46].

Not only bacterial but also fungal dysbiosis can occur in liver cirrhosis, highlighting the complexity of microbiota changes in such patients. Bacteroidetes/Ascomycota ratio can be used to predict 90-day hospitalization in patients with cirrhosis [47].

Hepatic encephalopathy

HE is a common complication in liver cirrhosis and the second most common decompensating event after ascites [48]. Despite therapeutic advances, HE patients still have poor survival. While the precise mechanism of HE remains unclear, the role of ammonia is most compelling. Ammonia is derived from urea breakdown by urease producing intestinal bacteria (predominantly gram-negative Enterobacteriaceae), and by glutamine deamidation by glutaminase [49, 50]. Hyperammonemia has been shown to worsen HE, whereas reducing blood ammonia improves HE, therefore, intestinal microbiota-targeted hyperammonemia correction can be an important therapeutic tool for patients with CLD. For the first time, the Consensus on Hyperammonemia in Adults was adopted in Russia in 2019 [51].

Primary sclerosing cholangitis

PSC is one of the autoimmune liver diseases, along with PBC and AIH, characterized by cholestasis, biliary inflammation, and stricturing. PSC is strongly associated with IBD, and the gut-liver axis with a gut microbiota as a key player contributes to PSC pathogenesis [52].

The first report of PSC-associated gut dysbiosis, independent from IBD, was published in 2016, suggesting a potential role of intestinal microbiota in the development and progression of PSC [53]. Dysbiosis in PSC was characterized by reduced microbiota diversity. *Enterococcus*, *Lactobacillus*, and *Fusobacterium* were overrepresented in patients with PSC, and an operational taxonomic unit (OTU) related to genus *Enterococcus* was positively correlated with serum levels of alkaline phosphatase (ALP), a disease severity marker. The same bacterial genera have already been associated with dysbiosis in liver cirrhosis [43, 46].

Subsequently, the independence of microbiota changes in PSC from associated ulcerative colitis was confirmed in a study that demonstrated a consistently increased abundance eight taxa in patients with PSC, including Proteobacteria, Gammaproteobacteria, Bacilli, Lactobacillales, *Parabacteroides* (bile-tolerant taxon involved in BA metabolism), *Streptococcus*, *Veillonella*, and an OTU assigned to *Bacteroides* [54]. The abundance of one OTU belonging to *Coprococcus*, as well as of other genera, comprising butyrate-producing species (*Faecalibacterium*, *Clostridium* IV), was markedly reduced in patients with PSC [54].

The features of mucosa-associated microbiota in PSC were the enrichment of *Blautia* and Barnesiellaceae and shifts in OTUs within Clostridiales and Bacteroidales orders [55]. Members of the last two taxa play an important role in the intestinal BA biotransformation, which may be associated with the pathogenesis of PSC.

A recent study showed for the first time an increased abundance of three intestinal pathobionts (*Klebsiella pneumoniae*, *Proteus mirabilis*, and *Enterococcus gallinarum*) in PSC patients, suggesting their involvement in the pathogenesis of disease through epithelial barrier disruption, initiation of bacterial translocation, and induction of Th17 cell-mediated immune response that drives liver inflammation [56]. This finding paves the way for targeted therapy of PSC.

As with cirrhosis, fungi also contribute to dysbiosis in PSC. A fungal gut dysbiosis in patients with PSC is characterized by increased biodiversity, increased abundance of *Exophiala*, and a decrease in *Saccharomyces cerevisiae*. In patients with PSC, both the bacterial and fungal signatures differed from those in patients with IBD. Disruption in the correlation network between bacteria and fungi in the microbiota of PSC patients was observed [57].

3.4 Microbiome and Liver Disease, continued.

Hepatocellular carcinoma and intestinal microbiome

Primary liver cancer, including HCC, which accounts for 75-80% of cases, tied for a 3-4th leading cause of worldwide cancer mortality [3]. Most cases of HCC develop in patients with liver cirrhosis caused by chronic viral hepatitis, NAFLD, and ARLD [58].

Possible mechanisms of the contribution of dysbiotic microbiota to liver carcinogenesis are derived predominantly from animal studies and include increased permeability, bacterial translocation, increased hepatic exposure to endotoxin, bacterial metabolites, and MAMPs, impaired BA metabolism with NKT cell involvement [58, 59]. MAMPs can provoke the senescence-associated secretory phenotype (SASP) in hepatic stellate cells (HSC), promote the secretion of hepatomitogen epiregulin, act on macrophages to trigger tumor-promoting inflammation, HSC activation and liver fibrosis [58].

The microbial signature in HCC may be like that in CLD, especially in cirrhosis. Specific signs of altered gut microbiota in patients with HCC include intestinal overgrowth of *Escherichia coli* in cirrhotic patients with HCC [60], enrichment in potential pro-inflammatory bacteria (*Escherichia/Shigella*, *Enterococcus*) and a decrease in SCFA-producing bacteria (*Faecalibacterium*, *Ruminococcus*, *Ruminoclostridium*) in patients with non-hepatitis virus-related HCC [61], a higher abundance of *Bacteroides* and Ruminococcaceae, and a reduction in *Bifidobacterium* in HCC with NAFLD-related cirrhosis [62]. In the latter study, the gut microbiota has been significantly correlated with systemic inflammation, suggesting their co-involvement in liver carcinogenesis.

Interestingly, fecal microbial diversity was decreased from healthy controls to cirrhosis, but it was increased from cirrhosis to early HCC with cirrhosis. In this study, Actinobacteria and 13 bacterial genera including *Gemmiger* and *Parabacteroides* were enriched in early HCC versus cirrhosis, LPS-producing bacteria were increased, while butyrate-producing bacteria were decreased in early HCC [63]. Gut microbial markers have demonstrated strong diagnosis potential for both early HCC advanced HCC, which may have translational significance in the management of patients with liver cancer.

Gut microbiota modulation as a promising therapeutic strategy in liver disease

Since the gut microbiome plays an important role in the development and progression of liver disease, it becomes an attractive therapeutic target. The main potential interventions that can modulate the microbiome include diet, probiotics (single-strain probiotics, multi-strain probiotics), prebiotics, non-absorbable disaccharides (lactulose, lactitol), synbiotics (a mixture of probiotics and prebiotics), postbiotics (microbial metabolites and/or bacterial cell-wall components), antibiotics, faecal microbiota transplantation (FMT), and phage cocktails (Table 1) [64].

Although diet, exercise, and lifestyle modification are widely used in the management of NAFLD patients, little is known about the effects of different diets on the altered gut microbiome. However, there is some evidence for the beneficial use of the Mediterranean diet rich in prebiotic dietary fiber in NAFLD [65] and the Middle Eastern diet in patients with cirrhosis [66]. The latter diet rich in fermented milk, vegetables, cereals, coffee, and tea is associated with increased microbial diversity, which in turn was associated with an independently lower risk of 90-day hospitalizations.

Diet
Probiotics (single-strain, multi-strain)
Prebiotics, dietary fiber, and non-absorbable disaccharides (lactulose, lactitol)
Synbiotics (a mixture of probiotics and prebiotics)
Postbiotics (microbial metabolites and/or bacterial cell-wall components)
Antibiotics
Faecal microbiota transplantation (FMT)
Phage cocktails

Table 1. *Potential interventions to modulate the gut microbiota in liver disease*

Probiotics can improve liver function tests – alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in patients with steatosis and steatohepatitis, lower the insulin resistance score (HOMA-IR), improve elastography

3.4 Microbiome and Liver Disease, continued.

(FibroScan), and even reduce histological activity in NASH. In an Italian study, a probiotic *Bifidobacterium longum* W11 with fructo-oligosaccharides (FOS) significantly reduced TNF-α, C-reactive protein, serum AST, HOMA-IR, serum endotoxin, improved steatosis, and decreased the NASH activity index [67].

In phase 1 randomized controlled trial (RCT), *Lactobacillus rhamnosus* GG significantly reduced the pathogenic taxa associated with cognitive impairment (Enterobacteriaceae, Porphyromonadaceae), and increased autochthonous taxa (Lachnospiraceae, Clostridiales XIV), increased CDR, and decreased endotoxemia and potentially ammoniagenic amino acids in cirrhosis with minimal HE [16].

In a pilot study, lyophilized *Saccharomyces boulardii* CNCM I-745 significantly reduced initially elevated fecal *Escherichia coli* in patients with NAFLD steatosis, thus reducing the potential risk of liver damage by endogenous ethanol. *S. boulardii* significantly lowered *Bacteroides fragilis* group, thus reducing the risk of endotoxemia. The lack of progression of steatosis after 90 days suggests the effectiveness of *S. boulardii* in NAFLD [68].

A recent double-blinded, randomized, placebo-controlled phase 2 trial showed that synbiotic treatment (FOS at 4 g twice a day plus *Bifidobacterium animalis* subsp. *lactis* BB-12 at a minimum of 10 billion colony-forming units/day) for approximately 12 months significantly improved the gut microbiota in patients with NAFLD [69]. Changes in microbiota after synbiotic treatment were characterized by increased β-diversity, increased abundance of beneficial *Bifidobacterium* and *Faecalibacterium*, and reduction of *Oscillibacter* and *Alistipes*, suggesting potential anti-inflammatory effects. However, this prebiotic treatment was ineffective in decreasing liver fat content or in improving validated biomarker scores for liver fibrosis or liver stiffness measurement. Thus, the improvement of the gut microbiota with the synbiotic occurred without any clinically significant hepatic effects in NAFLD patients.

There is little evidence to date on the effective use of prebiotics in patients with CLD [70], except for prebiotic non-absorbable disaccharides such as lactulose and lactitol. Cochrane review showed that the lactulose and lactitol were associated with beneficial effects on HE in patients with cirrhosis. Additional analyses showed that non-absorbable disaccharides can help to reduce serious adverse events associated with CLD including liver failure, hepatorenal syndrome, and variceal bleeding [71].

The inconclusive or conflicting results of some studies using probiotics and/or prebiotics suggest that novel, non-conventional candidate probiotic strains and some bacterial metabolites, such as butyrate and IPA, may be clinically effective in CLD. A deficiency in butyrate-producing bacteria, which is common in patients with liver disease, justifies the potential clinical use of butyrate-producing strains or commercial butyrate products (calcium butyrate plus inulin, tributyrin, etc.).

In an experimental animal study, butyrate-producing probiotic strain *Clostridium butyricum* MIYAIRI 588 and sodium butyrate were able to activate the hepatic adenosine 5'-monophosphate-activated protein kinase (AMPK), which inhibits hepatic lipogenesis, thereby preventing the progression of NAFLD [72]. Another candidate probiotic for NAFLD patients could be butyrate- and propionate-producing *Eubacterium hallii*, belonging to Lachnospiraceae. Oral *E. hallii* treatment was found to increase faecal butyrate levels and to modify BA metabolism in obese and diabetic *db/db* mice [73].

IPA, an intestinal bacterial tryptophan metabolite, modulated the gut microbiota composition and inhibited dysbiosis in rats fed a high-fat diet. IPA induced the expression of tight junction proteins (ZO-1 and occludin) and maintained intestinal epithelium homeostasis, leading to a reduction in endotoxemia [74].

Thus, though probiotics, prebiotics, synbiotics, and bacterial metabolites are seen as a potential therapeutic strategy for LCD, their beneficial effects should be further demonstrated by large RCT. Until now (then), only four probiotic/synbiotic products are recommended for use in patients with NAFLD/NASH, and three multi-strain probiotic products and one nonabsorbable disaccharide are recommended for use in HE by the World Gastroenterological Organization (WGO) [75].

Antibiotics are of limited use in CLD. Rifaximin, which has demonstrated therapeutic efficacy in hepatic encephalopathy [76], however, was not effective in patients with NASH

3.4 Microbiome and Liver Disease, continued.

in a UK open-label pilot study [77]. However, research on antibiotics is ongoing. In an Egyptian study, rifaximin was shown to be effective and safe in NASH patients by modifying the disease through the reduction of endotoxemia and improvement of insulin resistance, proinflammatory cytokines, cytokeratin-18 (CK-18), and NAFLD-liver fat score [78]. In another study from China, rifaximin ameliorated ascites and improved survival of cirrhotic patients with refractory ascites most likely by modulating the microbiota [79]. The use of vancomycin for PSC, which may have a positive effect through exposure to the gut microbiota with significant improvement in ALP, γ-glutamyl transferase (GGT), and Mayo PSC Risk Score (MRS), is being discussed [80, 81]. A significant disadvantage of antibiotics is that after antibiotic treatment, a significantly reduced microbial diversity, higher *Candida*, and lower relative abundance of autochthonous bacteria were seen [47]. Further research and large RCT are needed to establish if antibiotics are effective and safe CLD treatment.

Faecal or intestinal microbiota transplantation is a promising therapeutic strategy in patients with CLD. Unlike widespread studies of FMT in *Clostridioides difficile* infection, current trials of FMT in CLD are smaller and focus on the safety and structural and functional changes of the gut microbiota [82].

In an open-label RCT, FMT reduced hospitalizations, improved cognition, and dysbiosis in patients with cirrhosis with recurrent HE. None of the recipients developed HE within 5 months after FMT [83]. In a pilot study, FMT was effective and safe in patients with severe alcoholic hepatitis and improved liver disease severity and survival at 1 year. Microbiota analysis showed favorable changes in recipients after FMT at 1 year [84]. A recent, phase 1 RCT showed that FMT is safe and associated with a short-term reduction in alcohol craving and consumption with favorable microbial changes in patients with alcohol-related cirrhosis with alcohol misuse. There was a reduction in serum IL-6 and lipopolysaccharide-binding protein, increased microbial diversity with higher Ruminococcaceae and other SCFA-producing taxa, and increased butyrate/isobutyrate compared to baseline in FMT [85]. In an open-label pilot study in PSC patients with IBD, FMT safely increased bacterial diversity (including SCFA-producing taxa) that correlated with an improvement in ALP. 30% of PSC patients experienced a ≥50% decrease in ALP levels [86]. A study on FMT in patients with cirrhosis (PROFIT Trial, NCT02862249) was started. PROFIT trial differs from the previous ones in that the FMT is administered by upper GI endoscopy to deliver microbiota directly to the small bowel. The primary outcome measure will be safety and feasibility as assessed by recruitment rates, tolerability, and safety of FMT [87].

Another promising treatment is the phage cocktail that targets pathobionts strongly associated with some CLD. Currently, the first steps in the development of rationally designed phage cocktails targeting *Klebsiella pneumoniae* for PSC [56] and *Enterococcus faecalis* for alcoholic hepatitis are being taken [88, 89].

In summary, increasing evidence for a strong association between gut dysbiosis and liver disease paves the way for promising microbiome-based therapeutic, diagnostic, and prognostic strategies to be developed in the nearest future.

References

1. Asrani SK, et al. Burden of liver diseases in the world. *J Hepatol.* 2019;70(1):151–171.
2. GBD 2017 Cirrhosis Collaborators. The global, regional, and national burden of cirrhosis by cause in 195 countries and territories, 1990-2017: a systematic analysis for the Global Burden of Disease Study 2017. *Lancet Gastroenterol Hepatol.* 2020;5(3):245–266.
3. Bray F, et al. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin.* 2018;68(6):394–424.
4. Thomas DL. Global Elimination of Chronic Hepatitis. *N Engl J Med.* 2019;380(21):2041–2050.
5. Quigley EM, et al. The gut microbiota and the liver. Pathophysiological and clinical implications. *J Hepatol.* 2013;58(5):1020–1027.

3.4 Microbiome and Liver Disease, continued.

6. Zeng Y, et al. Gut microbiota dysbiosis in patients with hepatitis B virus-induced chronic liver disease covering chronic hepatitis, liver cirrhosis and hepatocellular carcinoma. *J Viral Hepat.* 2020;27(2):143–155.
7. Inoue T, et al. Gut dysbiosis associated with hepatitis C virus infection. *Clin Infect Dis.* 2018;67(6):869–877.
8. Woodhouse CA, et al. Review article: the gut microbiome as a therapeutic target in the pathogenesis and treatment of chronic liver disease. *Aliment Pharmacol Ther.* 2018;47(2):192–202.
9. Holzapfel WH, et al. Overview of gut flora and probiotics. *Int J Food Microbiol.* 1998;41(2):85–101.
10. Larsen PE, Dai Y. Metabolome of human gut microbiome is predictive of host dysbiosis. *Gigascience.* 2015;4:42.
11. Guinane CM, Cotter PD. Role of the gut microbiota in health and chronic gastrointestinal disease: understanding a hidden metabolic organ. *Therap Adv Gastroenterol.* 2013;6(4):295–308.
12. Zhou D, Fan JG. Microbial metabolites in non-alcoholic fatty liver disease. *World J Gastroenterol.* 2019;25(17):2019–2028.
13. Das B, Nair GB. Homeostasis and dysbiosis of the gut microbiome in health and disease. *J Biosci.* 2019;44(5):117.
14. Tripathi A, et al. The gut-liver axis and the intersection with the microbiome. *Nat Rev Gastroenterol Hepatol.* 2018;15(7):397–411.
15. Chu H, et al. Small metabolites, possible big changes: a microbiota-centered view of non-alcoholic fatty liver disease. *Gut.* 2019;68(2):359–370.
16. Acharya C, Bajaj JS. Altered microbiome in patients with cirrhosis and complications. *Clin Gastroenterol Hepatol.* 2019;17(2):307–321.
17. Chopyk DM, Grakoui A. Contribution of the Intestinal Microbiome and Gut Barrier to Hepatic Disorders. *Gastroenterology.* 2020:S0016-5085(20)34839-3.
18. Jiang W, et al. Dysbiosis gut microbiota associated with inflammation and impaired mucosal immune function in intestine of humans with non-alcoholic fatty liver disease. *Sci Rep.* 2015;5:8096.
19. Poeta M, et al. Gut-Liver Axis Derangement in Non-Alcoholic Fatty Liver Disease. *Children (Basel).* 2017;4(8):66.
20. Quigley EM, Monsour HP. The Gut Microbiota and Nonalcoholic Fatty Liver Disease. *Semin Liver Dis.* 2015;35(3):262–269.
21. Miele L, et al. Gut-liver axis and microbiota in NAFLD: insight pathophysiology for novel therapeutic target. *Curr Pharm Des.* 2013;19(29):5314–5324.
22. de Faria Ghetti F, et al. Influence of gut microbiota on the development and progression of nonalcoholic steatohepatitis. *Eur J Nutr.* 2018;57(3):861–876.
23. Romano KA, et al. Metabolic, Epigenetic, and Transgenerational Effects of Gut Bacterial Choline Consumption. *Cell Host Microbe.* 2017;22(3):279–290.e7.
24. Wang Z, et al. Gut flora metabolism of phosphatidylcholine promotes cardiovascular disease. *Nature.* 2011;472(7341):57–63.
25. Spencer MD, et al. Association between composition of the human gastrointestinal microbiome and development of fatty liver with choline deficiency. *Gastroenterology.* 2011;140(3):976–986.
26. Aron-Wisnewsky J, et al. Gut microbiota and non-alcoholic fatty liver disease: new insights. *Clin Microbiol Infect.* 2013;19(4):338–348.
27. Yu J, et al. The Pathogenesis of Nonalcoholic Fatty Liver Disease: Interplay between Diet, Gut Microbiota, and Genetic Background. *Gastroenterol Res Pract.* 2016;2016:2862173.
28. Quigley EM. Leaky gut – concept or clinical entity? *Curr Opin Gastroenterol.* 2016;32(2):74–79.
29. Wieland A, et al. Systematic review: microbial dysbiosis and nonalcoholic fatty liver disease. *Aliment Pharmacol Ther.* 2015;42(9):1051–1063.

3.4 Microbiome and Liver Disease, continued.

30. Shen F, et al. Gut microbiota dysbiosis in patients with non-alcoholic fatty liver disease. *Hepatobiliary Pancreat Dis Int.* 2017;16(4):375–381.
31. Loomba R, et al. Gut Microbiome-Based Metagenomic Signature for Non-invasive Detection of Advanced Fibrosis in Human Nonalcoholic Fatty Liver Disease. *Cell Metab.* 2017;25(5):1054–1062.e5.
32. Bajaj JS, et al. Altered profile of human gut microbiome is associated with cirrhosis and its complications. *J Hepatol.* 2014;60(5):940–947.
33. Wong VW, et al. Molecular characterization of the fecal microbiota in patients with nonalcoholic steatohepatitis–a longitudinal study. *PLoS One.* 2013;8(4):e62885.
34. Seliverstov PV, et al. Saccharomyces boulardii modulates the composition of the gut microbiota in patients with non-alcoholic fatty liver disease, thus preventing the progression of the disease. *Eksp Klin Gastroenterol.* 2018;(2):4–18.
35. Boursier J, et al. The severity of nonalcoholic fatty liver disease is associated with gut dysbiosis and shift in the metabolic function of the gut microbiota. *Hepatology.* 2016;63(3):764–775.
36. Fialho A, et al. Small Intestinal Bacterial Overgrowth Is Associated with Non-Alcoholic Fatty Liver Disease. *J Gastrointestin Liver Dis.* 2016;25(2):159–165.
37. Okushin K, et al. Helicobacter pylori infection and liver diseases: Epidemiology and insights into pathogenesis. *World J Gastroenterol.* 2018;24(32):3617–3625.
38. Chen C, et al. Helicobacter pylori infection may increase the severity of nonalcoholic fatty liver disease via promoting liver function damage, glycometabolism, lipid metabolism, inflammatory reaction and metabolic syndrome. *Eur J Gastroenterol Hepatol.* 2020;32(7):857–866.
39. Castaño-Rodríguez N, et al. NAFLD, Helicobacter species and the intestinal microbiome. *Best Pract Res Clin Gastroenterol.* 2017;31(6):657–668.
40. Bajaj JS. Alcohol, liver disease and the gut microbiota. *Nat Rev Gastroenterol Hepatol.* 2019;16(4):235–246.
41. Sarin SK, et al. Microbiome as a therapeutic target in alcohol-related liver disease. *J Hepatol.* 2019;70(2):260–272.
42. Dubinkina VB, et al. Links of gut microbiota composition with alcohol dependence syndrome and alcoholic liver disease. *Microbiome.* 2017;5(1):141.
43. Chen Y, et al. Characterization of fecal microbial communities in patients with liver cirrhosis. *Hepatology.* 2011;54(2):562–572.
44. Parada Venegas D, et al. Short Chain Fatty Acids (SCFAs)-Mediated Gut Epithelial and Immune Regulation and Its Relevance for Inflammatory Bowel Diseases. *Front Immunol.* 2019;10:277.
45. Bajaj JS. Altered Microbiota in Cirrhosis and Its Relationship to the Development of Infection. *Clin Liver Dis (Hoboken).* 2019;14(3):107–111.
46. Qin N, et al. Alterations of the human gut microbiome in liver cirrhosis. *Nature.* 2014;513(7516):59–64.
47. Bajaj JS, et al. Fungal dysbiosis in cirrhosis. *Gut.* 2018;67(6):1146–1154.
48. Bohra A, Worland T, Hui S, Terbah R, Farrell A, Robertson M. Prognostic significance of hepatic encephalopathy in patients with cirrhosis treated with current standards of care. *World J Gastroenterol.* 2020;26(18):2221–2231.
49. Jayakumar AR, Norenberg MD. Hyperammonemia in Hepatic Encephalopathy. *J Clin Exp Hepatol.* 2018;8(3):272–280.
50. Rai R, Saraswat VA, Dhiman RK. Gut microbiota: its role in hepatic encephalopathy. *J Clin Exp Hepatol.* 2015;5(Suppl 1):S29-36.
51. Lazebnik LB, et al. Russian Consensus "Hyperammonemia in Adults". *Eksp Klin Gastroenterol.* 2019;(12):4–23.

3.4 Microbiome and Liver Disease, continued.

52. Little R, et al. Gut microbiome in primary sclerosing cholangitis: A review. *World J Gastroenterol.* 2020;26(21):2768–2780.
53. Sabino J, et al. Primary sclerosing cholangitis is characterised by intestinal dysbiosis independent from IBD. *Gut.* 2016;65(10):1681–1689.
54. Rühlemann M, et al. Consistent alterations in faecal microbiomes of patients with primary sclerosing cholangitis independent of associated colitis. *Aliment Pharmacol Ther.* 2019;50(5):580–589.
55. Torres J, et al. The features of mucosa-associated microbiota in primary sclerosing cholangitis. *Aliment Pharmacol Ther.* 2016;43(7):790–801.
56. Nakamoto N, et al. Gut pathobionts underlie intestinal barrier dysfunction and liver T helper 17 cell immune response in primary sclerosing cholangitis. *Nat Microbiol.* 2019;4(3):492–503.
57. Lemoinne S, et al. Fungi participate in the dysbiosis of gut microbiota in patients with primary sclerosing cholangitis. *Gut.* 2020;69(1):92–102.
58. Schwabe RF, Greten TF. Gut microbiome in HCC – Mechanisms, diagnosis and therapy. *J Hepatol.* 2020;72(2):230–238.
59. Ma C, et al. Gut microbiome-mediated bile acid metabolism regulates liver cancer via NKT cells. *Science.* 2018;360(6391):eaan5931.
60. Grąt M, et al. Profile of Gut Microbiota Associated With the Presence of Hepatocellular Cancer in Patients With Liver Cirrhosis. *Transplant Proc.* 2016;48(5):1687–1691.
61. Liu Q, et al. Alteration in gut microbiota associated with hepatitis B and non-hepatitis virus related hepatocellular carcinoma. *Gut Pathog.* 2019;11:1.
62. Ponziani FR, et al. Hepatocellular Carcinoma Is Associated With Gut Microbiota Profile and Inflammation in Nonalcoholic Fatty Liver Disease. *Hepatology.* 2019;69(1):107–120.
63. Ren Z, et al. Gut microbiome analysis as a tool towards targeted non-invasive biomarkers for early hepatocellular carcinoma. *Gut.* 2019;68(6):1014–1023.
64. Quigley EMM. Microbiome Modulation in Liver Disease. *Clin Liver Dis (Hoboken).* 2019;14(4):149–151.
65. Abenavoli L, et al. Health benefits of Mediterranean diet in nonalcoholic fatty liver disease. *Expert Rev Gastroenterol Hepatol.* 2018;12(9):873–881.
66. Bajaj JS, et al. Diet affects gut microbiota and modulates hospitalization risk differentially in an international cirrhosis cohort. *Hepatology.* 2018;68(1):234–247.
67. Malaguarnera M, et al. Bifidobacterium longum with fructo-oligosaccharides in patients with non alcoholic steatohepatitis. *Dig Dis Sci.* 2012;57(2):545–553.
68. Seliverstov P, et al. FRI-266-Saccharomyces boulardii modulates the colonic microbiota towards a more favourable composition in patients with non-alcoholic fatty liver disease (simple steatosis). *J Hepatol.* 2019;70(1):e511.
69. Scorletti E, et al. Synbiotics Alter Fecal Microbiomes, But Not Liver Fat or Fibrosis, in a Randomized Trial of Patients With Nonalcoholic Fatty Liver Disease. *Gastroenterology.* 2020;158(6):1597–1610.e7.
70. Parnell JA, et al. The potential role of prebiotic fibre for treatment and management of non-alcoholic fatty liver disease and associated obesity and insulin resistance. *Liver Int.* 2012;32(5):701–711.
71. Gluud LL, et al. Non-absorbable disaccharides versus placebo/no intervention and lactulose versus lactitol for the prevention and treatment of hepatic encephalopathy in people with cirrhosis. *Cochrane Database Syst Rev.* 2016;2016(5):CD003044.
72. Endo H, et al. Butyrate-producing probiotics reduce nonalcoholic fatty liver disease progression in rats: new insight into the probiotics for the gut-liver axis. *PLoS One.* 2013;8(5):e63388.

3.4 Microbiome and Liver Disease, continued.

73. Udayappan S, et al. Oral treatment with Eubacterium hallii improves insulin sensitivity in db/db mice. *NPJ Biofilms Microbiomes.* 2016;2:16009.
74. Zhao ZH, et al. Indole-3-propionic acid inhibits gut dysbiosis and endotoxin leakage to attenuate steatohepatitis in rats. *Exp Mol Med.* 2019;51(9):1–14.
75. World Gastroenterology Organisation Global Guidelines 'Probiotics and Prebiotics'. 2017 Feb. Available at: http://www.worldgastroenterology.org/guidelines/global-guidelines/probiotics-and-prebiotics/probiotics-and-prebiotics-english [Accessed 21 August 2020].
76. Hudson M, Schuchmann M. Long-term management of hepatic encephalopathy with lactulose and/or rifaximin: a review of the evidence. *Eur J Gastroenterol Hepatol.* 2019;31(4):434–450.
77. Cobbold JFL, et al. Rifaximin in non-alcoholic steatohepatitis: An open-label pilot study. *Hepatol Res.* 2018;48(1):69–77.
78. Abdel-Razik A, et al. Rifaximin in nonalcoholic fatty liver disease: hit multiple targets with a single shot. *Eur J Gastroenterol Hepatol.* 2018;30(10):1237–1246.
79. Lv XY, Ding HG, Zheng JF, Fan CL, Li L. Rifaximin improves survival in cirrhotic patients with refractory ascites: A real-world study. *World J Gastroenterol.* 2020;26(2):199–218.
80. Shah A, et al. Effects of Antibiotic Therapy in Primary Sclerosing Cholangitis with and without Inflammatory Bowel Disease: A Systematic Review and Meta-Analysis. *Semin Liver Dis.* 2019;39(4):432–441.
81. Damman JL, et al. Review article: the evidence that vancomycin is a therapeutic option for primary sclerosing cholangitis. *Aliment Pharmacol Ther.* 2018;47(7):886–895.
82. Bajaj JS, Khoruts A. Microbiota changes and intestinal microbiota transplantation in liver diseases and cirrhosis. *J Hepatol.* 2020;72(5):1003–1027.
83. Bajaj JS, et al. Fecal microbiota transplant from a rational stool donor improves hepatic encephalopathy: A randomized clinical trial. *Hepatology.* 2017;66(6):1727–1738.
84. Philips CA, et al. Healthy Donor Fecal Microbiota Transplantation in Steroid-Ineligible Severe Alcoholic Hepatitis: A Pilot Study. *Clin Gastroenterol Hepatol.* 2017;15(4):600–602.
85. Bajaj JS, et al. A Randomized Clinical Trial of Fecal Microbiota Transplant for Alcohol Use Disorder. *Hepatology.* 2020.
86. Allegretti JR, et al. Fecal Microbiota Transplantation in Patients With Primary Sclerosing Cholangitis: A Pilot Clinical Trial. *Am J Gastroenterol.* 2019;114(7):1071–1079.
87. Woodhouse CA, et al. PROFIT, a PROspective, randomised placebo controlled feasibility trial of Faecal mIcrobiota Transplantation in cirrhosis: study protocol for a single-blinded trial. *BMJ Open.* 2019;9(2):e023518.
88. Kredo-Russo S., et al. 0026 Use of a bacteriophage cocktail for eradication of Klebsiella pneumoniae in primary sclerosing cholangitis. *Abstracts of the Liver Meeting of the American Association for the Study of Liver Diseases (AASLD), Boston.* 2019 Nov. Available at: https://plan.core-apps.com/tristar_aasld19/abstracts [Accessed 21 August 2020].
89. Duan Y, et al. Bacteriophage targeting of gut bacterium attenuates alcoholic liver disease. *Nature.* 2019;575(7783):505–511.

3.5 Microbiota and Esophagogastric Disorders

Richard H Hunt, FRCP, FACG, AGAF, MWGO

Master of American College of Gastroenterology
Master WGO
Professor Emeritus
Farncombe Family Digestive Health Research Institute
McMaster University, Hamilton, Ontario, Canada

Henry Cohen, MD, FACG, AGAF, MWGO

Master of American Gastroenterology
Former President WGO
Professor of Gastroenterology
Montevideo, Uruguay

Yessica Pontet, MD

Assistant of Gastroenterology
Hospital de Clínicas
Montevideo, Uruguay

Introduction

The constitution of the microbiota of the proximal digestive tract is currently the subject of extensive research, not only to know its diversity but also its potential pathogenic or indeed protective role. The composition of the microbiota includes not only bacteria but also fungi and viruses but most of our current understanding comes from bacterial populations in the stomach and to a lesser extent the esophagus. There is interest in how the microbiome could influence esophageal diseases, such as esophagitis and Barrett's esophagus and their progression to esophageal cancer. In the stomach much is already known about the relationship of *Helicobacter pylori* as a cause of gastritis and how colonization alters gastric physiology resulting in both duodenal and gastric ulcer and in some people gastric cancer. However, since the stomach contains gastric acid and digestive enzymes, it was long considered sterile and a surprisingly long time after the discovery of *H. pylori* by Marshall and Warren before the possibility of a gastric microbiome was considered. The remarkable ability of *H. pylori* to manipulate its' own microclimate supports the notion that other micro-organisms can also occupy the gastric mucosa. Indeed, modern techniques have identified hundreds of phylotypes with a lesser microbial density in the stomach of between 10^1 to 10^3 bacteria mL^{-1} of intestinal content than in the lower gastrointestinal tract where the concentration of microorganisms is around 10^{10}–10^{11} bacteria g^{-1} of intestinal content. There are other important considerations particular to the upper GI tract. Transit through the esophagus is rapid and in the stomach the residence time of ingested food or drink is short compared to the large bowel. To understand the dynamics of the microbiota the precise site and methods of sampling must be considered. For example, bacteria and DNA retrieved from luminal samples in the esophagus or stomach reveal very different patterns of bacteria compared with those from the mucosa. Importantly, microbes not only interact with the host but also with each other which can lead to significant microbial imbalance and dysbiosis.

Microbiota and the esophagus

The esophagus has long been considered sterile since it normally does not retain food, and any organisms isolated are likely to be of oropharyngeal origin and swallowed. Thus, the few microorganisms that have been identified were considered transient. *Candida, Cytomegalovirus* or *Herpes* infections have been linked to particular cases of esophagitis, predominantly in immunosuppressed patients.

Currently several publications have established that colonization of the esophagus can occur with the most common microorganism being *Streptococcus viridans*. This is consistent with findings in the oropharynx and it has been recovered from esophageal lavage, brushings and esophageal biopsies. Using 16S rDNA sequencing techniques it has been confirmed that the most common genera in biopsies of the distal esophagus are *Streptococcus*, *Prevotella* and *Veillonella*. The same findings were also confirmed by Fillon et al. by pyrosequencing, using a novel capsule called the Enterotest™.

3.5 Microbiota and Esophagogastric Disorders, continued.

Gastro-esophageal reflux disease

Gastro-esophageal reflux disease has the potential to change the esophageal epithelium, so a change in the scarce local flora might be expected. Osías et al. were the first to report that the overall esophageal bacterial concentration was directly associated with the severity of reflux-related esophagitis. In a more detailed study, Yang et al. identified in reflux esophagitis and also in Barrett's esophagus, that Gram negative bacteria predominate and included *Veillonella*, *Firmicutes*; *Bacteroidetes* (*Prevotella*), *Proteobacterias* (*Haemophilus*, *Neisseria*, and *Campylobacter*) and *Fusobacterias*. Liu et al. similarly described the increase in *Bacteroidetes* with a predominance of *Prevotella* and *Fusobacteria*. On the other hand, they noted a decrease in *Streptococcus* and *Proteobacteria* (with enrichment of *Neisseria*) and an increase in *Veillonella*.

Esophageal cancer

The change from Gram positive to Gram negative predominance of the esophageal flora induces a significant inflammatory response that triggers the dysbiosis cycle, inflammation and again dysbiosis. Furthermore, loss of bacterial diversity and atrophic gastritis is increasingly associated with distal esophageal cancer. Intriguingly, Narikiyo et al. reported cases of esophageal cancer that were associated with a periodontal infection with the spirochaete *Treponema denticola, and also Streptococcus mitis* and *anginosus,* all of which were found in esophageal mucosa and cancer and were resistant to host β-Defensins. These organisms are also associated with the production of proinflammatory cytokines associated with carcinogenesis. Higher levels of *Campylobacter concisus* and *Campylobacter rectus* have been found in patients with premalignant Barrett's esophagus, and could be linked with pathogenesis. Interestingly they are also more commonly found in periodontal infection and in enteritis.

Squamous carcinoma of the esophagus appears to be inversely related to the diversity of the esophageal flora and also, in a study from Iran, to atrophic fundus gastritis with increased *Clostridiales* and *Erysipeltrichales species* of the phylum Firmicutes associated with early squamous dysplasia and squamous cell cancer. *Porphyromonas gingivalis*, is another oral pathogen which it has been suggested is involved in the development and severity of squamous lesions.

Microbiota and the stomach

Historically the stomach was considered a hostile organ for microorganisms to survive. However, more than three decades ago a bacterium capable of adapting to the adverse environment was identified and now known as *Helicobacter pylori (Vide infra)*. The clinical and basic science research undertaken since then has provided us with an excellent basis and direction for the study of the gastric microbiome. With the development of new diagnostic methods, a number of other bacteria have been described in the gastric microbiome: *Enterococcus, Pseudomonas, Staphylococcus* and *Stomatococcus*. Subsequently, based on more specific sequencing methods, approximately 130 phylotypes corresponding to 7-8 classes have been identified and the most common genera are *Streptococcus, Prevotella, Veillonella* and *Rothia*. *Lactobacillus* species are found in the stomachs of all mammals and several studies have reported *Lactobacillus* species colonizing the human gastric mucosa. Micro-organisms common to the oral cavity have also frequently been reported including *Streptococcus genus* but many others contribute to the existence of a flora with its own characteristics.

Differences between the flora found in the gastric mucosa and the gastric juice are important. The flora of the gastric mucosa is greater and dominated by *Firmicutes* and *Proteobacteria*. The balance depends on age, diet, drug use, mucosal inflammation, and the presence or not of *Helicobacter pylori* infection. In contrast, the flora found in gastric fluid samples is more diverse, including oropharyngeal organisms which do not truly colonize the stomach and are mainly composed of Firmicutes, *Bacteroidetes* and *Actinobacteria*.

Helicobacter pylori

Helicobacter pylori is a Gram negative, bacillus and is present in the stomach of approximately half of the world population. It is the most studied gastric microorganism and is associated with gastritis, peptic ulcer, gastric adenocarcinoma, and MALT lymphoma. *H. pylori* has evolved complex mechanisms to colonize the stomach and control its own

3.5 Microbiota and Esophagogastric Disorders, continued.

microenvironment. These include bacterial urease which converts gastric urea to ammonia which in turn buffers gastric acid. Adhesion proteins (BabA adhesin) allow the bacteria to attach to surface gastric epithelial cells, which help to keep the bacteria in close apposition to the mucosa within the mucus layer at its most favourable pH. Adhesins also help the bacterium to avoid being swept away with each gastric emptying and provide warning of when the rapidly turning-over gastric cells are about to slough. *H. pylori* is flagellated and has a spiral shape which provide motility to optimize survival, location and colonization.

Lactobacilli

Lactobacilli are rod-shaped, gram-positive, micro-aerophilic bacteria and in several respects similar to *H. pylori.* In 1899 Jaworski described microscopic observations of human gastric juice in which he described spiral organisms which he named *Vibrio rugula* and short rods which he called *Lactococci.* He isolated the short rods and cultured them and they produced lactic acid thus meeting one of our criteria for *Lactobacillus*. The metabolism of this organism converts lactose to lactic acid, with consequent acidification of the bacterial micro-environment, the gastric mucous layer. Acidophilic gastric *Lactobacilli* adapt sufficiently to the acidic gastric environment and ca colonize the stomach due to these properties. In addition, some *Lactobacilli* possess a urease enzyme which has activity between pH 3-4, which is similar to that of *H. pylori*.

There is an interaction, which is at least theoretical and can be argued substantially on the basis of several findings. *Lactobacilli* produce lactic acid (0.25M - 0.50M) which acidifies the mucus and epithelial surface of the gastric antrum and thus inhibits gastrin release by G cells. In contrast *H. pylori* produce ammonia from urea, which alkalinizes the antral mucus and mucosal surface leading to hypergastrinemia. Furthermore, lactic acid at the concentration 0.25M - 0.50M also influences *H. pylori* bacteria by lowering cytoplasmic pH and reducing or inhibiting spontaneous growth.

Both *in vitro* and *in vivo* studies in animal models suggest that colonization mechanisms can be affected with the use of some probiotics such as those containing *Lactobacillus, Bifidobacterium* and *Saccharomyces*.

It has been suggested that probiotics could increase the eradication rate of therapeutic eradication regimens. They can certainly decrease antibiotic-related adverse effects. The impact on the gastric microbiota is unclear but it has been postulated that it could change the bacterial balance: while it might influence *Lactobacillus*, it would not likely affect concentrations of *Enterococcus, Staphylococcus aureus, Bifidobacterium* and *Bacteroides*. Current guidelines are either equivocal in not recommending the use of probiotics for this indication, or restricting prescribing to counter adverse events of antibiotic treatment.

Previously it had been described that in the stomach of those infected with *Helicobacter pylori,* it is the *Proteobacteria* and *Spirochetes* which predominate while in uninfected individuals the majority species were *Actinobacteria, Firmicutes*, and *Bacteroidetes*. Similar levels of *Proteobacterias*, *Firmicutes*, *Actinobacterias*, *Bacteroidetes* and *Fusobacterias* have recently been reported in both groups.

Dyspepsia and gastritis

Chronic *Helicobacter pylori* infection is the main cause of chronic atrophic gastritis. The mechanisms are multiple and include the stimulation of pro-inflammatory factors such as interleukin (IL) 1β and IL8, reactive oxygen species and the induction of apoptosis. Under these conditions, acid secretion decreases, and this is associated with an increase in the overall gastric abundance of bacteria. More recently reports indicate that there is less abundance and loss of bacterial diversity. A decrease in the concentrations of *Tannerella*, *Treponema* y *Prevotella* has also been described.

Lactobacillus can inhibit *Helicobacter pylori* adhesion and urease activity and thus regulate their interaction with epithelial cells and decreasing inflammation. Those belonging to *Bifidobacterium* resemble in their characteristics those of *Lactobacillus*. They have immunoregulatory activity and could inhibit Heli*cobacter pylori* infection and its consequences.

Autoimmune gastritis involves other mechanisms and there is little information on the gastric microbiota involved and what may initiate this pathological entity. Acid secretion is probably the most profoundly reduced physi-

3.5 Microbiota and Esophagogastric Disorders, continued.

ological function amongst the gastritides with the highest serum gastrin levels and high bacterial diversity with greater proportions of *Streptococcus* than other groups, with more *Firmicutes* than in patients with chronic atrophic gastritis. Patients with autoimmune gastritis also have *Ruminococcus* and *Gemella* which are not seen in other groups. Co-occurrence networks are disrupted by the large number of *Streptococcus* resulting in few connections. It is not known whether changes are due to a different immunity profile or the lack of gastric secretion.

Gastric cancer

Gastric cancer is the result of multifactorial pathological events which include genetics, diet and *Helicobacter pylori* infection. Bacterial diversity tends to decrease with the transition from non-atrophic gastritis to intestinal metaplasia and subsequently gastric cancer. There is a decrease in *Porphyromonas*, *Neisseria, TM7* group and *S. sinensis*, with a relative increase in *L. coleohominis* and Lachnospiraceae. It is not known whether other components of the gastric microbiota potentiate or inhibit the recognized effects of this bacteria although animal work has suggested that intestinal species augment and extend atrophy and dysplasia in the stomach. Dicksved et al. found no difference in gastric flora between those with gastric cancer and those with dyspepsia and a normal gastric mucosa. However, they did not employ a metagenomics-based strategy as others have reported.

This is a complicated area with potential confounders not always considered. Studies usually report findings at a single time point and often in the presence of established or advanced cancer when necrotic tissue is also present. Wang et al. found a predominance of *Proteobacterias, Firmicutes, Bacteroidetes, Fusobacterias* and *Actinobacterias*. For Hsieh et al., the most abundant were *Clostridium*, *Fusobacterium* and *Lactobacillus*. On the other hand, Ferreira et al. found that the microbiota in this context is less diverse, with less abundance of *Helicobacter pylori*.

Summary

The composition of the microbiome of the proximal digestive tract is complex and a subject of ongoing research activity and there is interest in how it could influence esophageal and gastric diseases beyond that which is *H. pylori* related. The most common microorganism of the esophagus is *Streptococcus viridans*. Reflux-related esophagitis is directly associated with bacterial concentration, especially Gram-negative organisms. The change from Gram positive to negative predominance is seen in Barrett's esophagus and cancer. Despite gastric acidity, stomach microbiota is more extensive and varied than in the esophagus. *Helicobacter pylori* is a well-known cause of gastritis, gastroduodenal ulcer and gastric cancer. Its presence alters the gastric bacterial diversity. Conversely, *Lactobacillus* can inhibit *Helicobacter pylori* adhesion and urease activity, decreasing inflammation. Among the multifactorial causes of gastric cancer, the role of the other microbiota is not yet clear and findings in this context are variable. However, advances in metagenomics and new research strategies will hopefully clarify the way forward in the next few years.

Further reading

Engstrand L, et al. Helicobacter pylori and the gastric microbiota. Best Pract Res Clin Gastroenterol. 2013; 27: 39–45.

Ferreira RM, et al. Gastric microbial community profiling reveals a dysbiotic cancer-associated microbiota. Gut. 2018; 67: 226–36.

Gagliardi D, et al. Microbial flora of the normal esophagus. Dis Esophagus 1998; 11: 248-50.

Hunt RH, Yaghoobi M. The Esophageal and Gastric Microbiome in Health and Disease. *Gastroenterol Clin North Am*. 2017;46(1):121-141. doi:10.1016/j.gtc.2016.09.009

Jandhyala, S.M. Role of the normal gut microbiota. World J. Gastroenterol. 2015; 21: 8787–8803.

Jaworski W. Podrêcznik chorób zoladka (Handbook of Gastric Diseases). Wydawnictwa Dziel Lekarskich Polskich, 1899: 30-47.

Li XX, et al. Bacterial microbiota profiling in gastritis without Helicobacter pylori infection or non-steroidal anti-inflammatory drug use. PLoS One 2009; 4: e7985.

Liu, N, et al. Characterization of bacterial biota in the distal esophagus of Japanese patients with reflux esophagitis and Barrett's esophagus. BMC Infect. Dis. 2013; 13: 130.

3.5 Microbiota and Esophagogastric Disorders, continued.

Parsons BN, et al. Comparison of the human gastric microbiota in hypochlorhydric states arising as a result of Helicobacter pylori-induced atrophic gastritis, autoimmune atrophic gastritis and proton pump inhibitor use. PLoS Pathog. 2017; 13: e1006653.

Sharma BK, et al. Intragastric bacterial activity and nitrosation before, during, and after treatment with omeprazole. Brit Med J. 1984; 289: 717–19.

Sohn SH, et al. Analysis of gastric body microbiota by pyrosequencing: possible role of bacteria other than Helicobacter pylori in the gastric carcinogenesis. J Cancer Prev. 2017; 22: 115–25.

Wang ZK, et al. Upper gastrointestinal microbiota and digestive diseases. World J Gastroenterol 2013; 19 (10): 1541-50.

Dicksved J, Lindberg M, Rosenguist M, Enroth H, Jansson JK, Engstrand L. Molecular characterization of the stomach microbiota in patients with gastric cancer and in controls. J Med Microbiol 2009; 58: 509-16.

Fillon SA, Harris JK, Wagner BD, Kelly CJ, Stevens MJ, Moore W, et al. Novel device to sample the esophageal microbiome--the esophageal string test. PLoS One 2012; 7: e42938.

Hsieh YY, Tung SY, Pan HY, et al. Increased abundance of Clostridium and Fusobacterium in gastric microbiota of patients with gastric cancer in Taiwan. Sci Rep. 2018; 8: 158.

Narikiyo, M., C. Tanabe, Y. Yamada, et al. Frequent and preferential infection of Treponema denticola, Streptococcus mitis, and Streptococcus anginosus in esophageal cancers. Cancer Sci. 2004; 95: 569–74.

Osias, G.L., M.Q. Bromer, R.M. Thomas, et al. Esophageal bacteria and Barrett's esophagus: a preliminary report. Dig. Dis. Sci. 2004; 49: 228–36.

Yang L, Lu X, Nossa CW, Francois F, Peek RM, Pei Z. Inflammation and intestinal metaplasia of the distal esophagus are associated with alterations in the microbiome. Gastroenterology 2009; 137: 588-97.

4.1 Probiotics: the Concept

Mary Ellen Sanders, PhD

Executive Science Officer
International Scientific Association for Probiotics and Prebiotics
Littleton, Colorado, USA

What is a probiotic?

Probiotics are live microorganisms that, when administered in adequate amounts, confer a health benefit on the host.[1] In practice, this definition for probiotic also requires that it be: (a) defined to the genus, species and strain levels according to current nomenclature and using current methods; (b) safe for its intended use; (c) supported by adequate evidence from at least one well-designed human trial that aligns with any claim made, including comparable study population (applicable to the intended user of the product), study outcomes and the study dose in the target host demonstrating a health benefit; and (d) maintained alive in the product in a dose sufficient to convey its health benefits, all the way through its shelf life (see Binda et al. 2020 for a review of probiotic criteria). Isolated microbial endproducts, preparations providing mainly dead microbes, or undefined microbes fall outside the scope of probiotics.

The probiotic 'umbrella' is broad, covering different types of microbes, routes of administration, target hosts, health effects and regulatory categories (Figure). Probiotics have uses in companion animals and in animal and plant agriculture, and may be used as animal feed additives or as inoculants on plants or in soil. This chapter is restricted to probiotics for human use. See Table 1 for some related definitions. Probiotics span several different regulatory categories, for example, conventional food, infant formula, medical foods, dietary (nutritional) supplements, and drugs (also known as live biotherapeutic agents). Probiotics may

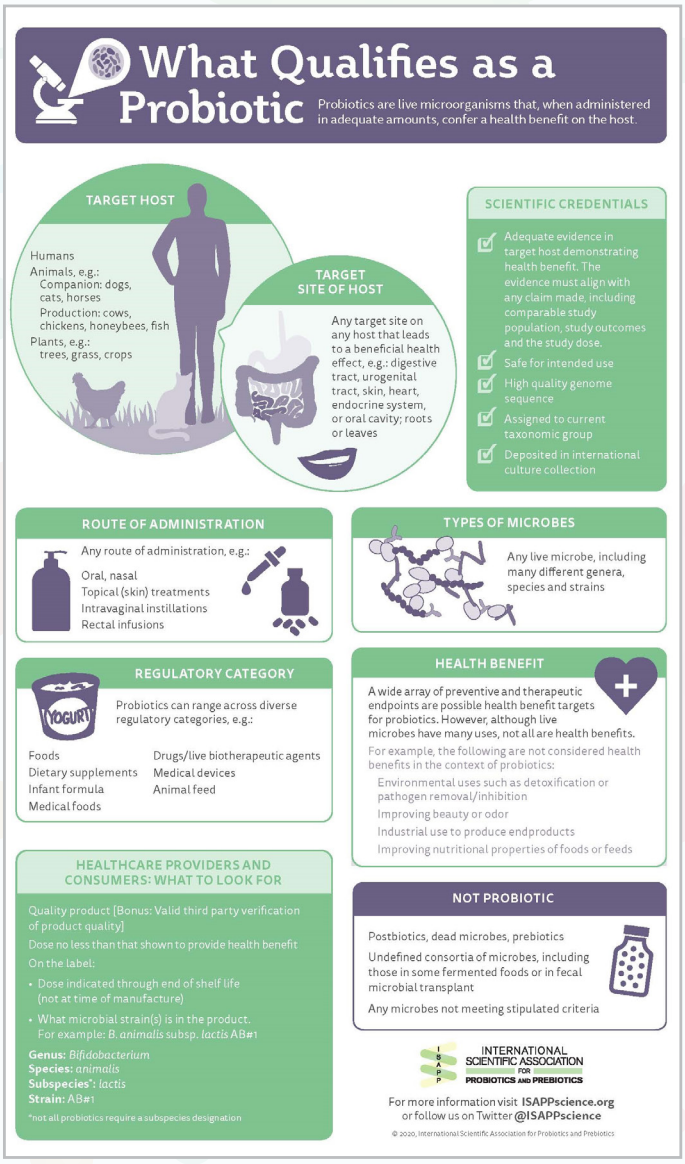


Figure. The scope of probiotics. Copyright 2020, International Scientific Association for Probiotics and Prebiotics, used with permission. Available here.

1 This definition was initially proposed by an Expert Consultation convened by the FAO/WHO in 2001, and refined by a panel of experts convened by ISAPP in 2013 (Hill et al. 2014).

4.1 Probiotics: the Concept, continued.

Probiotic	Live microorganisms that when administered in adequate amounts confer a health benefit on the host. Hill et al. 2014
Live cultures	Microbes primarily used for the fermentation of foods. Health benefits of these microbes may not have been tested, and therefore, these are not considered to be 'probiotics'
Fermented Food	Foods made through desired microbial growth and enzymatic conversions of food components
Prebiotic	A substrate that is selectively utilized by host microorganisms conferring a health benefit. Gibson et al. 2017
Synbiotic	A mixture comprising live microorganisms and substrate(s) selectively utilized by host microorganisms that confers a health benefit on the host. Swanson et al. 2020
Postbiotic	Proposed definition: A preparation of inanimate microorganisms and/or their components that confers a health benefit on the target host. Salminen et al. Under review.

Table 1. Definitions

also be included in products that are not administered orally, such as topical skin treatments, nasal sprays, intravaginal instillations or rectal infusions. Demonstration of a health benefit for a probiotic is a cornerstone requirement, but a wide array of endpoints is possible, including different target sites or organs of the body, and many possible physiological, disease or quality of life readouts.

Microbes used as probiotics

Several different genera and species of bacteria and yeast are used as probiotics. The most common are species within the family *Lactobacillaceae* or the genus *Bifidobacterium* (*B. adolescentis, B. animalis, B. bifidum, B. breve and B. longum*). The genus *Lactobacillus* was recently reorganized and species once under this large (over 250 species), heterogeneous genus are now spread over 25 genera, including 23 novel genera. All new genus names for species containing existing probiotic strains still begin with the letter 'L'. *L. casei, L. fermentum, L. paracasei, L. plantarum, L. rhamnosus* and *L. salivarius* all have new genus names; see here for a tool to find new genus names. *L. acidophilus, L. crispatus* and *L. delbreuckii* remain in the *Lactobacillus* genus. Other probiotics include strains of *Saccharomyces cerevisiae* var. *boulardii* (a yeast), *Escherichia coli* and *Bacillus coagulans*. Newly identified human commensal bacteria associated with health properties may comprise probiotics of the future. Examples of microbes being considered for such use include *Akkermansia muciniphila*, *Faecalibacterium prausnitzii*, *Roseburia* spp. and *Eubacterium hallii*.

In addition to many different genera and species of probiotics, different strains of the same species are also used. Strain designations are assigned by the researchers or marketers of the specific strain, and there are no conventions for such names. For example, for the probiotic strain *Lactobacillus acidophilus* NCFM, the genus is "*Lactobacillus*", the species is "*acidophilus*" and the strain designation is "NCFM."

Health benefits

Other chapters in this manual explore specific benefits of probiotics. However, a few recent papers summarize probiotic health effects, including Merenstein et al. 2020, Sanders et al. 2018, and Su et al. 2020. Several intestinal and extra-intestinal benefits have been associated with probiotic administration, including reduced risk of atopic dermatitis/food hypersensitivity, reduced incidence of and prevention of morbidity and mortality associated with necrotizing enterocolitis, treatment of acute pediatric diarrhea, management of symptoms of occasional constipation, management of symptoms of lactose intolerance, reduced incidence and duration of common infectious diseases (upper respiratory tract and gastrointestinal), reduced risk of antibiotic-associated diarrhea, extended remission of ulcerative colitis, improved therapeutic efficacy of antibiotic treatment of bacterial vaginosis, reduced low-density lipoprotein cholesterol and reduced risk of *Clostridioides difficile* diarrhea. Effect sizes and number needed to treat vary for conditions.

It should be noted that many effects are not broadly distributed among all probiotics. To the extent that different

4.1 Probiotics: the Concept, continued.

strains may confer similar benefits, pooling data on strains with mechanistic similarity is useful to consider the totality of evidence, although analysis of data for a single strain is preferred. The strength of conclusions from meta-analyses is impacted by heterogeneity among the included studies with regard to measurement of endpoints, subjects, probiotic strain and dose. In most cases, additional, well-controlled studies are needed to strengthen the evidence.

From a clinical perspective, it is important to realize that not all preparations called "probiotic" will have the same health effects. For example, research by O'Mahony et al. in 2005 compared the ability of *Ligilactobacillus salivarius* UCC4331 (formerly *Lactobacillus salivarius* UCC4331) or *Bifidobacterium longus* subsp. *longum* 35624 (formerly *B. infantis* 35624) to alleviate symptoms of irritable bowel syndrome; only strain 35624 was effective. Therefore, it is best to recommend probiotics that have been specifically tested and shown to have the desired benefits for the specific condition.

Probiotic effects are also dose-specific. Few dose response studies have been conducted on clinical endpoints, but some products are effective at 100 million colony-forming units (CFUs)/day whereas others are effective at more than 1 trillion CFU/day. This huge range in effective doses likely reflects differences in strains, clinical endpoints, and perhaps the best guess of the researcher of what level would be sufficient, as few dose response studies have been conducted. Therefore, it is best to recommend the dose of a specific probiotic that has been tested and shown to have desired benefits.

Safety

Probiotics must be safe under the intended conditions of their use. For different types of foods, including infant formula, and dietary supplements, probiotics must be safe when consumed by the generally healthy, age-appropriate population. For drugs, safety considers a risk/benefit assessment. It is important for clinicians to consider safety for off-label uses for probiotics, especially if administering to severely ill or immunocompromised patients. D-lactate producing probiotics are not recommended for patients with short bowel syndrome. Use of probiotics in either diseased or immunocompromised individuals must be done mindfully. However, many controlled studies have reported no serious, product-related adverse events in unhealthy or at-risk subjects, such as very low birth weight infants, patients with chronic inflammatory bowel diseases, intensive care unit patients, and patients with acute infectious diarrhea. Successful outcomes to such studies suggest that the identical product could be used with similar subjects, dose and route of administration under medical supervision. However, a report (Vallabhaneni et al. 2015) of an infant death from mucormycosis resulting from a probiotic contaminated with a mold serves as an important reminder that product quality must be assured and sufficient for the use (Sanders et al. 2016). Another risk with probiotic use is catheter line contamination from dispensing powdered probiotics in hospitalized patients. When in doubt, the product manufacturer should be able to provide guidance as to the type and extent of safety assessments that have been conducted on its product. Further, third party verification of product quality would assure users of compliance with applicable regulatory standards (Jackson et al. 2019).

Probiotic products

Choosing from the many different probiotic products can be challenging. Sources of some probiotic recommendations are provided in Table 2. Especially of note is the WGO Practice Guideline on Probiotics and Prebiotics. Tables 8 and 9 within this document summarize strength of evidence for specific probiotic strains and doses for particular gastrointestinal indications.

Probiotic product labels should disclose the genus, species, and strain designation of each probiotic strain contained in the product (see infographics here and here for example labels in USA and EU, respectively). This approach provides a level of confidence that the manufacturer is formulating the product with specific strains consistently over time. Furthermore, strain designations tie the product content back to scientific publications that document claimed health effects. The product label should also indicate the number of live (viable) microorganisms that are delivered in each serving or dose, and this level should be guaranteed throughout the expiration date. Levels are typically communicated as colony forming units, or CFUs, derived from culture-dependent plating methods, which is a mea-

4.1 Probiotics: the Concept, continued.

Source	Recommendation	Reference
World Gastroenterology Organisation	Comprehensive list of gastrointestinal endpoints that summarizes strength of evidence for specific probiotic strains (Tables 8 and 9)	WGO Practice Guideline - Probiotics and Prebiotics
American Gastroenterological Association	Prevention of NEC, sepsis and all-cause mortality in preterm, low birthweight infants Prevention of CDI for adults and children receiving antibiotic therapy	AGA Clinical Practice Guidelines on the Role of Probiotics in the Management of Gastrointestinal Disorders
Journal of Family Practice	Treat acute pediatric diarrhea Reduced incidence AAD Reduced incidence CDI Reduced symptoms of colic in breastfed infants Management f constipation Reduced symptoms associated with lactose maldigestion Improved therapeutic efficacy of antibiotic treatment of bacterial vaginosis	Probiotics as a Tx resource in primary care
European Society of Paediatric Gastroenterology, Hepatology and Nutrition	The use of probiotics for the management of acute gastroenteritis	Use of Probiotics for the Management of Acute Gastroenteritis in Children. An Update
	Prevention of NEC, sepsis and all-cause mortality in preterm, low birthweight infants	Probiotics for Preterm Infants: A Strain-Specific Systematic Review and Network Meta-analysis
	Prevention of pediatric nosocomial diarrhea	Probiotics for the Prevention of Nosocomial Diarrhea in Children
	Prevention of pediatric AAD	Probiotics for the Prevention of Antibiotic-Associated Diarrhea in Children

Table 2. Recommendations for clinical use for probiotics. AAD, antibiotic associated diarrhea; NEC, necrotizing enterocolitis; CDI, *C. difficile* infection.

sure of viable probiotics. Enumeration methods based on flow cytometry, reported in active fluorescent units (AFU), are emerging and may be used commercially as well, but before adoption of this new technology, a clear relationship between AFUs and CFUs, the measure used to enumerate probiotics in human trials to date, must be established.

The suggested serving size or dose should be indicated. Proper storage conditions and corporate contact information (including a website or consumer hotline number where additional information can be obtained) should be indicated. Finally, to the extent allowed by local regulatory authorities, labels should describe health benefits that have been substantiated in the target population for the product. Medical professionals need to be aware, however, that regulations limit the nature of what types of benefits can be described on food and dietary supplement products. Therefore, studies that refer to the treatment of a disease, in reducing side effects of drugs, promoting remission of a disease, or improving therapeutic efficacy of a drug may be precluded by regulatory authorities on labels for foods or dietary supplements, regardless of the strength of the

4.1 Probiotics: the Concept, continued.

evidence. Therefore, product labels might only list very general benefits.

Probiotic foods or nutritional supplements are sometimes disparaged as 'unregulated.' Although it is true that drugs are more closely regulated with regard to premarket substantiation of safety and efficacy, it is not correct that probiotic foods and supplements are unregulated. Regulation of these products varies by country or political region, but often good manufacturing practices and truthful labeling are required by law. Enforcement of these laws may be uneven, however, and premarket approval of safety and efficacy is not always a requirement. Indeed, commercial probiotic products may not be sufficiently labeled with strain designations and potency through the end of shelf life (Merenstein et al. 2019; Dailey et al. 2020). This information is necessary for consumers or healthcare providers to link products to research on efficacy.

Supplementary Reading and References:

1. Binda S, Hill C, Johansen E, Obis D, Pot B, Sanders ME, Tremblay A, Ouwehand A. 2020. Criteria to Qualify Microorganisms as "Probiotic" in Foods and Dietary Supplements. Front Microbiol. 24 July 2020.
2. Dailey Z, Sanders ME, Merenstein D. 2020. Retail refrigerated probiotic foods and their association with evidence of health benefits. Benef Microbes. 2020 Mar 27;11(2):131-133. doi: 10.3920/BM2019.0162. Epub 2020 Mar 25. PMID: 32208926
3. Hill C, Guarner F, Reid G, Gibson GR, Merenstein DJ, Pot B, Morelli L, Canani RB, Flint, HJ, Salminen S, Calder PC, Sanders ME. 2014. The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. Nat Rev Gastroenterol Hepatol 11:506-514. Sanders M.E., Akkermans LMA., Haller D, Hammerman C, Heimbach J, Huys G, Levy D, Mack D, Phothirath P, Constable A, Solano-Aguilar G, Vaughan E. 2010. Assessment of probiotic safety for human use. Gut Microbes 1 (3):1-22. http://www.landesbioscience.com/journals/gutmicrobes/article/12127/
4. ISAPP infographics and videos that discuss the basics of probiotics, prebiotics and fermented foods developed by the academic board members of ISAPP at https://ISAPPscience.org/for-consumers/infographics/ and https://ISAPPscience.org/for-consumers/videos/.
5. Jackson SA, Schoeni JL, Vegge C, Pane M, Stahl B, Bradley M, Goldman VS, Burguière P, Atwater JB, Sanders ME. Improving End-User Trust in the Quality of Commercial Probiotic Products. Front Microbiol. 2019 Apr 17;10:739. doi: 10.3389/fmicb.2019.00739
6. Jinshui Zheng J, Wittouck S, Salvetti E, Franz CMAP, Harris HMB, Mattarelli P, O'Toole PW, Pot B, Vandamme P, Walter J, Watanabe K, Wuyts S, Felis GE, Gänzle MG, Lebeer S. A taxonomic note on the genus Lactobacillus: Description of 23 novel genera, emended description of the genus Lactobacillus Beijerinck 1901, and union of Lactobacillaceae and Leuconostocaceae. Int J Syst Evol Microbiol. 2020 Apr;70(4):2782-2858.
7. Marco ML, Sanders ME, Gänzle M, Arrieta MC, Cotter PD, De Vuyst, L, Hill C, Holzapfel W, Lebeer S, Merenstein D, Reid G, Wolfe BE, Hutkins R. Expert consensus document: The International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on fermented foods. Nat Rev Gastroenterol Hepatol. In Press
8. Merenstein DJ, Guzzi J, Sanders ME. 2019. More Information Needed on Probiotic Supplement Product Labels. J Gen Intern Med. 34(12):2735-2737. DOI: 10.1007/s11606-019-05077-5
9. Merenstein DJ, Sanders ME, Tancredi DJ. Probiotics as a Tx resource in primary care. J Fam Pract. 2020 Apr;69(3):E1-E10.
10. O'Mahony L, McCarthy J, Kelly P, Hurley G, Luo F, Chen K, O'Sullivan GC, Kiely B, Collins JK, Shanahan F, Quigley EM. Lactobacillus and bifidobacterium in irritable bowel syndrome: symptom responses and relationship to cytokine profiles. Gastroenterology. 2005 Mar;128(3):541-51. doi: 10.1053/j.gastro.2004.11.050.

4.1 Probiotics: the Concept, continued.

11. Sanders ME, Merenstein DJ, Merrifield CA, Hutkins R. 2018. Probiotics for human use. Nutrition Bulletin 43(3): 212-225.

12. Sanders ME, Merenstein DJ, Ouwehand AC, Reid G, Salminen S, Cabana MD, Paraskevakos G, Leyer G. Probiotic use in at-risk populations. J Am Pharm Assoc (2003). 2016 Nov-Dec;56(6):680-686. doi: 10.1016/j.japh.2016.07.001.

13. Su GL, Ko CW, Bercik P, Falck-Ytter Y, Sultan S, Weizman AV, Morgan RL. AGA Clinical Practice Guidelines on the Role of Probiotics in the Management of Gastrointestinal Disorders. Gastroenterology. 2020 Jun 9;S0016-5085(20)34729-6.

14. Vallabhaneni S, Walker TA, Lockhart SR, Ng D, Chiller T, Melchreit R, Brandt ME, Smith RM; Centers for Disease Control and Prevention (CDC). Notes from the field: Fatal gastrointestinal mucormycosis in a premature infant associated with a contaminated dietary supplement--Connecticut, 2014. MMWR Morb Mortal Wkly Rep. 2015 Feb 20;64(6):155-6.

4.2 Probiotics in Pediatrics

Hania Szajewska, MD

Professor and Chair
Department of Paediatrics
The Medical University of Warsaw
Warsaw, Poland

Introduction

A 2014 definition developed by the International Scientific Association for Probiotics and Prebiotics (ISAPP), defines probiotics as *'live microorganisms that, when administered in adequate amounts, confer a health benefit on the host'* (1). The most commonly used probiotics are bacteria from the genus *Lactobacillus* or *Bifidobacterium*, and a yeast, *Saccharomyces boulardii*. However, novel probiotics (e.g. *Akkermansia, Faecalibacterium)* are an area of current investigation.

The health benefits of probiotics in children have been the subject of extensive research. Here, evidence from the latest meta-analyses of randomized controlled trials (RCTs) on the efficacy of probiotics in pediatrics is summarized. If available, most recent recommendations made by recognized scientific societies such as the European Society for Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN), the North American Society of Pediatric Gastroenterology, Hepatology and Nutrition (NASPGHAN), and the American Gastroenterology Association (AGA) are presented. For a summary of the clinical effects of probiotics in children, see **Table**.

Probiotics in Infant Formulas

Probiotics have been added to many infant formulas with the aim of shifting the microbiota to match that of a breast-fed infant with potential benefits attributed to breastfeeding. Infants fed such formulae are exposed to a daily intake of probiotic strains. This is in contrast to older children and adults in whom consumption of a probiotic product constitutes only a portion of their diets. Hence, both safety and efficacy are crucial. A 2017 systematic review concluded that the administration of so far evaluated probiotic-supplemented formulae to healthy infants does not raise safety concerns with regard to growth and adverse effects (2). Whereas some beneficial clinical effects of probiotics are possible, there is no existing robust evidence to recommend their routine use. The latter conclusion may reflect the small amount of data on a specific probiotic strain(s) and outcomes, rather than a genuine lack of an effect. These conclusions were in line with a 2011 position paper of the Committee on Nutrition of the ESPGHAN which, at least at that time, did not support the routine use of probiotic-supplemented formulae in infants (3). Similarly, according to a 2014 position of the European Food Safety Authority (EFSA) there is no necessity to add probiotics to infant and follow-on formulae (4).

Treatment of Acute Gastroenteritis

Until 2020, many, if not all, professional societies advocated use of probiotics with documented efficacy for the management of acute gastroenteritis. Currently, the recommendations differ, possibly reflecting negative (null) studies questioning the efficacy of some strains with previous positive recommendations.

In 2020, the ESPGHAN Working Group (WG) on Probiotics made weak (conditional) recommendations *for*: *S boulardii* (low to very low certainty of evidence); *L rhamnosus GG* (very low certainty of evidence); *L reuteri* DSM 17938 (low to very low certainty of evidence); and *L rhamnosus* 19070-2 & *L reuteri* DSM 12246 (very low certainty of evidence). The WG made a strong recommendation *against L helveticus* R0052 & *L rhamnosus* R0011 (moderate certainty of evidence) and a weak (conditional) recommendation *against Bacillus clausii* strains O/C, SIN, N/R, and T (very low certainty of evidence)(5).

In contrast, also in 2020, the AGA made a conditional recommendation *against* the use of probiotics in children from North America with acute infectious gastroenteritis (moderate quality of evidence)(6).

Prevention of Antibiotic-Associated Diarrhea (AAD)

For preventing AAD, in 2016, the ESPGHAN WG on Probiotics recommended using *L rhamnosus* GG (moderate quality

4.2 Probiotics in pediatrics, continued.

of evidence, strong recommendation) or *S boulardii* (moderate quality of evidence, strong recommendation). Other strains or combinations of strains have been tested, but sufficient evidence is still lacking. If the use of probiotics for preventing *Clostridioides difficile*-associated diarrhea is considered, the ESPGHAN WG suggested using *S boulardii* (low quality of evidence, conditional recommendation)(7).

In contrast, the AGA (2020) did not formulate any recommendations with regard to the use of probiotics for preventing AAD. However, the AGA conditionally recommended (based on low quality of evidence) certain probiotics for the prevention of *C difficile* infection in children receiving antibiotic treatment (for details, see **Table**)(6).

Respiratory Tract Infections

A number of systematic reviews reported that probiotics use was associated with reduced number and/or duration of respiratory tract infections, antibiotic courses used, and days absent from school (8-12). At the strain level, probiotics such as *L rhamnosus* GG or *L reuteri* DSM 17938 may have a modest effect on community-acquired respiratory infections in young children attending day-care centers (10). However, repeat studies are still needed. Explicit *for* or *against* recommendations have not been formulated.

Prevention of Allergy

In 2015, the World Allergy Organization (WAO) (13) concluded that probiotic supplementation cannot be recommended for reducing the risk of allergy in children. However, the WAO considered that there is a likely a net benefit from using probiotics for preventing eczema. Specifically, the WAO suggested: *'a) using probiotics in pregnant women at high risk for having an allergic child; b) using probiotics in women who breastfeed infants at high risk of developing allergy; and c) using probiotics in infants at high risk of developing allergy.'* All recommendations were conditional and supported by a very low quality of evidence. The recommendations were based on the findings of a 2015 meta-analysis which pooled data on all probiotics (14). Similarly, a 2018 meta-analysis, which also pooled data of all probiotics, showed that oral supplementation with probiotics during late pregnancy and lactation may reduce the risk of eczema (15). However, a 2018 strain-specific meta-analysis of RCTs showed that *L rhamnosus* GG, the only probiotic studied in more than one RCT, was ineffective in reducing eczema (16). This systematic review did not support the general recommendation to use probiotics for preventing eczema, unless specific strains would be indicated. Overall, while probiotics may be effective, it remains unclear which probiotic(s) should be used to reduce the risk of eczema.

Prevention of Necrotizing Enterocolitis

In 2020, both ESPGHAN (17) and AGA (6) published their recommendations on the use of probiotics for preventing NEC. While both were based on pair-wise systematic reviews and network meta-analyses, their conclusions differ. For details, see **Table**. The only probiotic strain that was recommended by both societies is *L rhamnosus* GG.

Helicobacter pylori Infection

According to 2017 ESPGHAN/NASPGHAN *H pylori* guidelines (18), the routine addition of either single or combination probiotics to eradication therapy to reduce side effects and/or improve eradication rates is currently not recommended. This is in contrast to the recommendations in adults (19).

Inflammatory Bowel Disease

In line with 2018 evidence-based guidelines by the ECCO and ESPGHAN, a mixture of eight strains [*L paracasei* DSM 24733, *L plantarum* DSM 24730, *L acidophilus* DSM 24735, *L delbrueckii* subspecies *bulgaricus* DSM 24734, *B longum* DSM 24736, *B infantis* DSM 24737, *B breve* DSM 24732, and *Str thermophilus* DSM 24731], or *Escherichia coli* Nissle 1917 may be considered as an effective treatment for maintenance in patients with mild ulcerative colitis as an adjuvant therapy or in those intolerant to 5-ASA; however, this recommendation is based on limited evidence (20).

In line with 2018 guidelines of the ESPGHAN Porto IBD Group (21), there is limited evidence in favor of using the mixture of eight strains (as above) or *L reuteri* ATCC 55730 as an adjuvant to standard therapy for induction of remission in mild-to-moderate pediatric ulcerative colitis. The mixture of eight strains has also shown efficacy for maintaining remission and possibly preventing pouchitis in adults, but data in children are lacking.

4.2 Probiotics in pediatrics, continued.

For Crohn's disease, according to the same 2018 ESPGHAN guidelines, there is not enough evidence to suggest that probiotics are beneficial for the induction or maintenance of remission of Crohn's disease in children (21).

The AGA (2020), both in patients with ulcerative colitis and Crohn's disease, recommends *against* the use of probiotics, unless in the context of a clinical trial (6). However, in adults and children with pouchitis, the AGA conditionally recommends the use of the 8-strain combination [as listed earlier] over no or other probiotics.

Infantile Colic

A 2018 individual participant data meta-analysis documented that the administration of *L reuteri* DSM 17938 is likely to reduce crying and/or fussing time in breastfed infants with infantile colic, but its role in formula-fed infants is less clear (22). For preventing infantile colic, a 2019 Cochrane review (23) found a similar occurrence of new cases of colic in the probiotics and placebo groups. However, probiotics (pooled together), reduced duration in crying time at study end. At the strain level, the effect was particularly evident for *L reuteri* DSM 17938. The same strain is likely to prevent crying in infants (24).

Functional Abdominal Pain Disorders

A 2018 systematic review concluded that there is insufficient evidence for the use of probiotics in children with functional abdominal pain disorders (25). There are no specific recommendations from ESPGHAN or NASPGHAN. The AGA 2020 guidelines noted with regard to IBS that there are many studies; however, significant heterogeneity in study design, outcomes, and probiotics used resulted in no recommendations for the use of probiotics in symptomatic children and adults with IBS (except in the context of a clinical trial).

Functional Constipation

Two recent systematic reviews (25, 26) do not support the use of probiotics for treating children with functional constipation. The findings of both systematic reviews support current ESPGHAN/NASPGHAN recommendations that probiotics should not be used in the treatment of functional constipation in children (27).

Other Diseases

A number of RCTs have evaluated various probiotics for preventing or treating other diseases. Both positive and negative (null) studies have been published. With few exception (e.g., pancreatitis for which probiotics are not recommended), for most of these diseases, explicit *for* or *against* recommendations have not been formulated. Among others, the other conditions for which probiotics have been studied include autism spectrum disorders; caries; celiac disease and non-celiac gluten sensitivity; cystic fibrosis; eczema; non-celiac gluten sensitivity; non-alcoholic fatty liver disease; pancreatitis; small bowel bacteria overgrowth, and type 1 diabetes.

SAFETY OF PROBIOTICS

Overall, probiotics are considered safe for use in otherwise healthy populations. Risk factors for adverse events include immunosuppression; prematurity; critical illness; presence of structural heart disease; hospitalization; presence of a central venous catheter; and the potential for translocation of probiotics across the bowel wall.

CONCLUSIONS

Probiotics have the potential to prevent and treat many disorders in the pediatric population. However, guidance is needed regarding which microorganism(s) to use for which clinical condition, as well as the timing, dosage, and mode of administration. Not all probiotics are equal. The clinical effects and safety of any single probiotic or combination of probiotics should not be extrapolated to other probiotics. It is reasonable to use the regimens proven to be effective in well-designed and executed RCTs in a given population. The use of products with no documented health benefits should be discouraged.

4.2 Probiotics in pediatrics, continued.

Table. Effects of probiotics in children

Condition	Society	Recommendation
Treatment of acute gastroenteritis	ESPGHAN 2020	Conditional (weak) recommendation *for* • *S boulardii* (250–750 mg/day, for 5–7 days) (low to very low certainty of evidence) • *L rhamnosus* GG (≥10^{10} CFU/day, typically 5–7 day) (very low certainty of evidence) • *L reuteri* DSM 17938 (1 x 10^8 to 2 x 10^8 to 4 x 10^8 CFU/day, for 5 days) (low to very low certainty of evidence) • *L rhamnosus* 19070–2 and *L reuteri* DSM 12246 (2 x 10^{10} CFU of each strain/d, for 5 days) (very low certainty of evidence) Strong recommendation *against* • *L helveticus* R0052 and *L rhamnosus* R0011 (moderate certainty of evidence) Weak recommendation *against* • *Bacillus clausii* O/C, SIN, N/R, and T (very low certainty of evidence).
	AGA 2020	Against the use of probiotics in children with acute infectious gastroenteritis in North America (conditional recommendation, moderate quality of evidence)
Prevention of antibiotic-associated diarrhea	ESPGHAN 2016	Strong recommendation *for* • *L rhamnosus* GG (moderate quality of evidence) • *S boulardii* (moderate quality of evidence)
	AGA 2020	Not addressed
Prevention of *C difficile* diarrhea	ESPGHAN 2016	*S boulardii* (weak recommendation; moderate quality of evidence)
	AGA 2020	• *S boulardii*; • A two-strain combination of *L acidophilus* CL 1285 & *L casei* LBC80R; • A three-strain combination of *L acidophilus, L delbruekii* subsp. *bulgaricus*, and *B bifidum*;* • A four-strain combination of *L acidophilus, L delbruekii* subsp. *bulgaricus, B bifidum*, and *Streptococcus salivarius* subsp. *thermophilus*.*
Allergy (prevention)	WAO 2015	WAO suggests the use of probiotics in select high-risk populations to reduce the risk of eczema; however, there is no clear indication regarding which probiotic(s) to use.
Respiratory tract infections		Not addressed

4.2 Probiotics in pediatrics, continued.

Prevention of necrotizing enterocolitis	ESPGHAN 2020	Conditional recommendation *for* • *L rhamnosus* GG ATCC53103 (at a dose ranging from 1x 10^9 CFU to 6x10^9 CFU) (low certainty of evidence). • *B infantis* Bb-02, *B lactis* Bb-12, and *Str thermophilus* TH-4 at 3.0 to 3.5×10^8 CFU (of each strain) (low certainty of evidence). No recommendation *for* or *against* • *L reuteri* DSM 17938 (very low certainty of evidence). • *B bifidum NCDO 1453 & L acidophilus NCDO 1748* (very low certainty of evidence). Conditional recommendation *against* • *B breve BBG-001* • *S boulardii*
	AGA 2020	Combination of *Lactobacillus* spp. and *Bifidobacterium* spp.: • *L rhamnosus* ATCC 53103 and *B longum* subsp. infantis;* • *L casei* and *B breve*;* • *L rhamnosus*, *L acidophilus*, *L casei*, *B longum* subsp. *infantis*, *B bifidum*, and *B longum* subsp. *longum*;* • *L acidophilus* and *B longum* subsp. *infantis*;* • *L acidophilus* and *B bifidum*;* • *L rhamnosus* ATCC 53103 and *B longum* Reuter ATCC BAA-999; • *L acidophilus*, *B bifidum*, *B animalis* subsp. *lactis*, and *B longum* subsp. *longum*;* • *B animalis* subsp *lactis* (including DSM 15954), • *L reuteri* (DSM 17938 or ATCC 55730), • *L rhamnosus* (ATCC 53103 or ATCA07FA or LCR 35)
H pylori infection	ESPGHAN & NASPGHAN 2017	Not recommended
Crohn's disease	ESPGHAN 2018	Not recommended
	AGA 2020	*Against* the use of probiotics, unless in the context of a clinical trial.
Ulcerative colitis	ESPGHAN & ECCO 2018	A mixture of 8 strains# or *Escherichia coli* Nissle 1917
	AGA 2020	*Against* the use of probiotics, unless in the context of a clinical trial.
Pouchitis	ESPGHAN 2018	A mixture of 8 strains#
	AGA 2020	A mixture of 8 strains#
Functional abdominal pain disorders, including irritable bowel syndrome (IBS)	ESPGHAN or NASPGHAN	No addressed
	AGA 2020	IBS. Only in the context of a clinical trial.

4.2 Probiotics in pediatrics, continued.

Infantile colic	ESPGHAN or NASPGHAN or AGA	Not addressed
Functional constipation	ESPGHAN & NASPGHAN 2014	Not recommended

AGA, American Gastroenterology Association; CFU, colony-forming units; ECCO, European Crohn's and Colitis Organization; ESPGHAN, European Society for Paediatric Gastroenterology, Hepatology and Nutrition; NASPGHAN, North American Society for Pediatric Gastroenterology, Hepatology and Nutrition; WAO, World Allergy Organization.

* No strain specification was given for any of the strains.

L paracasei DSM 24733, *L plantarum* DSM 24730, *L acidophilus* DSM 24735, *L delbrueckii* subspecies *bulgaricus* DSM 24734, *B longum* DSM 24736, *B infantis* DSM 24737, *B breve* DSM 24732, and *Str thermophilus* DSM 247]

REFERENCES

1. Hill C, Guarner F, Reid G, Gibson GR, Merenstein DJ, Pot B, et al. Expert consensus document. The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. Nature reviews Gastroenterology & hepatology. 2014;11(8):506-14.
2. Skorka A, Piescik-Lech M, Kolodziej M, Szajewska H. Infant formulae supplemented with prebiotics: Are they better than unsupplemented formulae? An updated systematic review. The British journal of nutrition. 2018;119(7):810-25.
3. Braegger C, Chmielewska A, Decsi T, Kolacek S, Mihatsch W, Moreno L, et al. Supplementation of infant formula with probiotics and/or prebiotics: a systematic review and comment by the ESPGHAN committee on nutrition. Journal of pediatric gastroenterology and nutrition. 2011;52(2):238-50.
4. Szajewska H, Szajewski T. Saturated Fat Controversy: Importance of Systematic Reviews and Meta-analyses. Crit Rev Food Sci Nutr. 2016;56(12):1947-51.
5. Szajewska H, Guarino A, Hojsak I, Indrio F, Kolacek S, Orel R, et al. Use of Probiotics for the Management of Acute Gastroenteritis in Children. An Update. Journal of pediatric gastroenterology and nutrition. 2020.
6. Su GL, Ko CW, Bercik P, Falck-Ytter Y, Sultan S, Weizman AV, et al. AGA Clinical Practice Guidelines on the Role of Probiotics in the Management of Gastrointestinal Disorders. Gastroenterology. 2020.
7. Szajewska H, Canani RB, Guarino A, Hojsak I, Indrio F, Kolacek S, et al. Probiotics for the Prevention of Antibiotic-Associated Diarrhea in Children. Journal of pediatric gastroenterology and nutrition. 2016;62(3):495-506.
8. Hao Q, Dong BR, Wu T. Probiotics for preventing acute upper respiratory tract infections. The Cochrane database of systematic reviews. 2015(2):Cd006895.
9. Wang Y, Li X, Ge T, Xiao Y, Liao Y, Cui Y, et al. Probiotics for prevention and treatment of respiratory tract infections in children: A systematic review and meta-analysis of randomized controlled trials. Medicine (Baltimore). 2016;95(31):e4509.
10. Laursen RP, Hojsak I. Probiotics for respiratory tract infections in children attending day care centers-a systematic review. Eur J Pediatr. 2018;177(7):979-94.

4.2 Probiotics in pediatrics, continued.

11. King S, Glanville J, Sanders ME, Fitzgerald A, Varley D. Effectiveness of probiotics on the duration of illness in healthy children and adults who develop common acute respiratory infectious conditions: a systematic review and meta-analysis. The British journal of nutrition. 2014;112(1):41-54.
12. Amaral MA, Guedes G, Epifanio M, Wagner MB, Jones MH, Mattiello R. Network meta-analysis of probiotics to prevent respiratory infections in children and adolescents. Pediatr Pulmonol. 2017;52(6):833-43.
13. Fiocchi A, Pawankar R, Cuello-Garcia C, Ahn K, Al-Hammadi S, Agarwal A, et al. World Allergy Organization-McMaster University Guidelines for Allergic Disease Prevention (GLAD-P): Probiotics. The World Allergy Organization journal. 2015;8(1):4.
14. Cuello-Garcia CA, Brozek JL, Fiocchi A, Pawankar R, Yepes-Nunez JJ, Terracciano L, et al. Probiotics for the prevention of allergy: A systematic review and meta-analysis of randomized controlled trials. The Journal of allergy and clinical immunology. 2015;136(4):952-61.
15. Garcia-Larsen V, Ierodiakonou D, Jarrold K, Cunha S, Chivinge J, Robinson Z, et al. Diet during pregnancy and infancy and risk of allergic or autoimmune disease: A systematic review and meta-analysis. PLoS Med. 2018;15(2):e1002507.
16. Szajewska H, Horvath A. Lactobacillus rhamnosus GG in the Primary Prevention of Eczema in Children: A Systematic Review and Meta-Analysis. Nutrients. 2018;10(9).
17. van den Akker CHP, van Goudoever JB, Shamir R, Domellöf M, Embleton ND, Hojsak I, et al. Probiotics and Preterm Infants: A Position Paper by the European Society for Paediatric Gastroenterology Hepatology and Nutrition Committee on Nutrition and the European Society for Paediatric Gastroenterology Hepatology and Nutrition Working Group for Probiotics and Prebiotics. Journal of pediatric gastroenterology and nutrition. 2020;70(5):664-80.
18. Jones NL, Koletzko S, Goodman K, Bontems P, Cadranel S, Casswall T, et al. Joint ESPGHAN/NASPGHAN Guidelines for the Management of Helicobacter pylori in Children and Adolescents (Update 2016). Journal of pediatric gastroenterology and nutrition. 2017;64(6):991-1003.
19. Malfertheiner P, Megraud F, O'Morain CA, Gisbert JP, Kuipers EJ, Axon AT, et al. Management of Helicobacter pylori infection-the Maastricht V/Florence Consensus Report. Gut. 2017;66(1):6-30.
20. Turner D, Ruemmele FM, Orlanski-Meyer E, Griffiths AM, de Carpi JM, Bronsky J, et al. Management of Paediatric Ulcerative Colitis, Part 1: Ambulatory Care-An Evidence-based Guideline From European Crohn's and Colitis Organization and European Society of Paediatric Gastroenterology, Hepatology and Nutrition. Journal of pediatric gastroenterology and nutrition. 2018;67(2):257-91.
21. Miele E, Shamir R, Aloi M, Assa A, Braegger C, Bronsky J, et al. Nutrition in Pediatric Inflammatory Bowel Disease: A Position Paper on Behalf of the Porto Inflammatory Bowel Disease Group of the European Society of Pediatric Gastroenterology, Hepatology and Nutrition. Journal of pediatric gastroenterology and nutrition. 2018;66(4):687-708.
22. Sung V, Cabana MD, D'Amico F, Deshpande G, Dupont C, Indrio F, et al. Lactobacillus reuteri DSM 17938 for managing infant colic: protocol for an individual participant data meta-analysis. BMJ Open. 2014;4(12):e006475.
23. Ong TG, Gordon M, Banks SS, Thomas MR, Akobeng AK. Probiotics to prevent infantile colic. Cochrane Database Syst Rev. 2019;3:Cd012473.
24. Ong TG, Gordon M, Banks SS, Thomas MR, Akobeng AK. Probiotics to prevent infantile colic. The Cochrane database of systematic reviews. 2019;3(3):Cd012473.

4.2 Probiotics in pediatrics, continued.

25. Wegh CAM, Benninga MA, Tabbers MM. Effectiveness of Probiotics in Children With Functional Abdominal Pain Disorders and Functional Constipation: A Systematic Review. Journal of clinical gastroenterology. 2018;52 Suppl 1, Proceedings from the 9th Probiotics, Prebiotics and New Foods, Nutraceuticals and Botanicals for Nutrition & Human and Microbiota Health Meeting, held in Rome, Italy from September 10 to 12, 2017:S10-s26.

26. Wojtyniak K, Szajewska H. Systematic review: probiotics for functional constipation in children. Eur J Pediatr. 2017;176(9):1155-62.

27. Tabbers MM, DiLorenzo C, Berger MY, Faure C, Langendam MW, Nurko S, et al. Evaluation and treatment of functional constipation in infants and children: evidence-based recommendations from ESPGHAN and NASPGHAN. Journal of pediatric gastroenterology and nutrition. 2014;58(2):258-74.

4.3 Fecal Microbiota Transplantation (FMT)

Dina Kao, MD

Associate Professor
Division of Gastroenterology, Department of Medicine
University of Alberta
Edmonton, Alberta, Canada

Tarkan Karakan, MD

Professor
Division of Gastroenterology, Department of Medicine
Gazi University
Ankara, Turkey

Introduction

The concept of FMT (also known as stool transplant or fecal microbial transfer) dates back to the 4th century in China. FMT was called "yellow soup" and was used to treat a variety of gastrointestinal illnesses. In 1958 Dr. Ben Eiseman gave FMT to 4 patients with pseudomembranous colitis and saw beneficial results. At that time, though, the organism *Clostridioides difficile* (formerly known as *Clostridium difficile*) had not been identified. *C. difficile* infection (CDI) is the classic example of how disruption of intestinal microbiota (dysbiosis) can lead to disease. CDI is most often caused by exposure to antibiotics that indiscriminately kill both harmful and harmless bacteria. Without the presence of beneficial commensal bacteria, *C. difficile* produces toxins A and B. The result is diarrhea, abdominal pain, and in rare cases death. Most patients with CDI do well after treatment for the infection, but 10% to 20% of them will have recurrent infections. Treating CDI with antibiotics such as vancomycin can perpetuate a vicious cycle of intestinal dysbiosis. For these patients with recurrent CDI (rCDI), FMT has become the standard-of-care therapy because there is no other effective treatment. FMT has had dramatic success in treating rCDI, and the perception is that it is "natural", not a drug. This has led to much interest in exploring FMT for other conditions related to intestinal dysbiosis, such as ulcerative colitis, irritable bowel syndrome, and metabolic syndrome.

FMT for treating rCDI

Clinical efficacy, access to treatment, and mechanisms of action

The overall efficacy of FMT for rCDI is at least 80% with a single treatment. Processed stool from a donor can be delivered by upper routes (e.g. nasogastric tube, gastroscopy, capsules) or lower routes (enema or colonoscopy). The landmark first random ized trial, done in the Netherlands, compared FMT delivered by nasogastric tube to vancomycin plus bowel preparation or vancomycin alone. The rate of success (no recurrence) was 94% in the FMT group of patients after 1–2 FMTs and 31% in the vancomycin group.[1] Later randomized trials showed similar efficacy for FMT delivered by colonoscopy[2] or by oral capsules of frozen stool.[3] Efficacy of fresh and frozen stool is similar.[4] For each treatment, using at least 50 g of donor stool is generally recommended because the success rate may diminish below this threshold. However, the best route to deliver FMT and the ideal formulation or dose remain to be determined. The decision on how to administer FMT currently depends on local expertise, resources, and availability.

Establishing stool banks became possible because frozen stool does not decrease clinical efficacy, and efficacy is similar for stool from patient-directed and anonymous donors. Stool banks can use processed stool from unrelated volunteer donors. Donations can be stored frozen at -80 °C for up to a year. The ideal model is to hold donor material in quarantine between screening visits to decrease the risk of potential disease transmission.[5] Stool banks dramatically improve access to FMT treatment. Many academic institutions have created their own small-scale stool banks where public stool banks may not exist. More recently, lyophilized (freeze dried) FMT has also shown clinical efficacy that is similar to fresh or frozen FMT.[6] Lyophilized FMT can make treatment even more convenient because this formulation may not require long-term storage at -80 °C .

How FMT works is still uncertain. What is well established by analyzing microbial composition is that rCDI patients

4.3 Fecal microbiota transplantation (FMT), continued.

have lower microbial diversity, increased abundance of bacteria from the phylum Proteobacteriacea, and reduced abundance of bacteria from Firmicutes and Bacteroidetes. After successful FMT, patients had their microbial diversity and their relative abundances of Firmicutes and Bacteroidetes increase to resemble the pattern in their donors. FMT is thus proposed to re-establish patients' resistance to colonization by *C. difficile*.

Other potential mechanisms of action have been proposed. FMT may restore metabolism of bile acids[7] and short chain fatty acids.[8] Some species of commensal bacteria convert primary bile acids (which promote *C. difficile* growth and germination) to secondary bile acids (which reduce *C. difficile* growth and germination). Stool samples from rCDI patients before FMT have shown reduced secondary bile acid levels. These levels significantly increase after successful FMT, to levels that resemble stool from healthy donors. After successful FMT, similar change is seen in levels of short chain fatty acids such as acetate, butyrate, and propionate. These short chain fatty acids are by-products of bacterial fermentation. They are the primary energy source for colonocytes (cells on the surface of the large intestine) and have effects on the immune system of the human host. Many species of bacteria also produce bacteriocin and quorum sensing molecules that can modulate microbial composition. Furthermore, many of these bacterial products and cell wall components can affect the immune system of the human (see Figure 1).

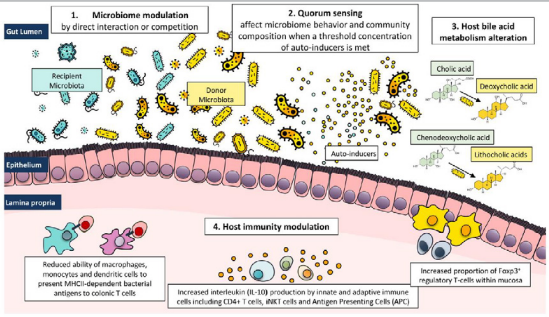


Figure 1. Potential mechanisms of FMT include direct interaction or competition between donor and recipient gut microbiota to achieve homeostasis, effect of donor microbiota on the host immunity and effect of microbiota on modulating host metabolism and physiology. Adapted from Ng SC et al. *Gut* 2020; 69: 83–91

Figure 1.

Recipients

Several CDI treatment guidelines recommend FMT after the second recurrence of CDI following the primary infection (i.e. 3rd episode). The timeline for a typical recurrence is 2–4 weeks, or up to 8 weeks after a course of antibiotic treatment directed at CDI. After an episode of CDI and anti-CDI treatment (e.g. vancomycin, metronidazole, fidaxomicin), diarrhea should be completely resolved. If not, an alternative cause for the diarrhea should be sought. A significant portion of patients referred for FMT due to suspected rCDI are reported to have a different cause for their symptoms, such as post infectious irritable bowel syndrome or inflammatory bowel disease. Careful assessment of patients before FMT is crucial to ensure that rCDI is the correct diagnosis.

Donors

Because most of the harmless microbes in the human gut cannot yet be grown in the lab, FMT requires healthy stool donors. Meticulous donor selection and screening is essential to minimize transmission of infectious agents or of donor traits linked to dysbiosis. A potential donor should be excluded if they have a history of high-risk behaviors, gastrointestinal illness such as inflammatory bowel disease, irritable bowel syndrome, autoimmune or neurological disorders, immune compromise, obesity, or cancer. At minimum, laboratory tests should rule out viral hepatitis, HIV, syphilis, and enteric pathogens such as *E. coli, Salmonella, Campylobacter*, and *C. difficile*. The testing needed for a potential donor is still under debate and may depend on local expertise, available resources, and relevance. Table 1 lists suggested tests for donor screening. Screening intervals may be set by local regulatory bodies or authorities, but are generally recommended to be 8 weeks or less. Detailed records of donor-recipient pairing are crucial for traceability if a donor develops a condition that can be transmitted through FMT.

Safety

FMT has few reported serious adverse events. Transient fever, abdominal pain, bloating, constipation, and flatus are the most common symptoms after FMT. More serious adverse events were reported recently in the USA: cyto-

4.3 Fecal microbiota transplantation (FMT), continued.

At minimum	
Serological	Stool
• Hepatitis A virus IgM • Hepatitis B surface antigen • Antibody to hepatitis C virus • HIV types 1 and 2 • Rapid plasma reagin for syphilis	• Culture for enteric pathogens • Ova and parasite examination • *C. difficile*
Consider if appropriate	
• Cytomegalovirus • Epstein-Barr virus • Human T-cell lymphoma virus	• Rotavirus • Norovirus • Adenovirus • *Vibrio* • Cryptosporidium • Microsporidia • Vancomycin-resistant enterococcus • Methicillin-resistant *Staphylococcus aureus* • Extended spectrum beta lactamase-producing *E. coli* • COVID-19

Table 1. Screening tests for stool donors

megalovirus colitis from self-administered FMT, death in an immunocompromised recipient from *E. coli* that produce extended spectrum beta lactamases, and diarrhea from *E. coli* that produce Shiga toxin. An asymptomatic donor may also shed viral particles such as norovirus or COVID-19, or may carry antibiotic resistance genes. We do not currently know the implications of these for FMT recipients. The long-term safety profile of FMT is even less well defined, with potential risks of developing chronic disease. A careful, detailed discussion about potential risks and benefits of FMT must be part of the informed consent process.

FMT for other gastrointestinal (GI) disorders

Given the high success rates of FMT in treating rCDI, it is very tempting to extrapolate results to other conditions linked to dysbiosis, such as inflammatory bowel disease (ulcerative colitis, Crohn's disease). This is a recurring inflammation of the intestine. Ulcerative colitis is restricted to the large intestine, but Crohn's disease can affect the entire gastrointestinal tract. Both have been linked to dysbiosis of the gut microbiota, with decreased microbial diversity and decreases in Bacteroidetes and Firmicutes. It is not clear whether dysbiosis is a cause or a consequence of inflammatory bowel disease.

However, the response rate to FMT for inflammatory bowel disease is much lower than for rCDI. A systematic review summarized 53 published studies that demonstrate clinical remission after FMT: 36% (201/555) of patients with ulcerative colitis, 51% (42/83) of patients with Crohn's disease and 22% (5/23) of patients with pouchitis. Four randomized trials of FMT treatment for ulcerative colitis have been published. Cochrane meta-analysis demonstrated a significantly higher clinical remission at 8 weeks in the FMT arm than in the control arm, with 37% (52/140) of FMT patients and 18% (24/137) of control patients achieving remission.[9] Evidence for FMT to treat Crohn's disease is even less robust. In a meta-analysis of 11 uncontrolled observational cohort studies and case series, 51% (42/83) of patients with Crohn's disease achieved clinical remission. A prospective study observed clinical remission in 52% (13/25) of patients with Crohn's disease 3 months after FMT. This decreased to 23% (5/22) of patients 18 months after FMT. A second FMT administered within 4 months of the first one maintained clinical benefits. The largest prospective study showed clinical remission in 57% (79/139) of patients with Crohn's disease 1 month after FMT, with favorable safety. However, the potential of FMT to treat Crohn's disease is still uncertain and well-designed controlled studies are needed.

The impacts of FMT on irritable bowel syndrome and chronic constipation are being investigated, but 2 randomized trials show conflicting results. In the first study, 65% (36/55) of patients in the FMT group had relief of irritable bowel syndrome symptoms (>75 points reduction in severity score) 3 months after a single FMT via colonoscopy, compared to 43% (12/28) of patients in the placebo group. The second study reported a larger reduction in severity score (-125.71) in the placebo group (23 patients) after 3 months than in the FMT group (22 patients) that received FMT capsules for 12 days (-52.45).[10] Although microbial

4.3 Fecal microbiota transplantation (FMT), continued.

diversity increased in the patients receiving FMT capsules, their symptoms did not clinically improve. Differences in study outcomes might arise from the different strategies to administer FMT or from a heterogenous population of patients with irritable bowel syndrome.

FMT regulation

Stool is a complex mixture. The exact composition in FMT is not known and will vary even for the same donor on different days. This is a significant challenge for regulatory agencies, because stool is unlike any other therapeutic approved for clinical use. Currently there is no consensus on how to classify or regulate FMT. In Canada and the USA it is regulated as a drug, and in Australia as a biologic. FMT remains unregulated in many countries. In North America, a treating physician can offer FMT to patients with rCDI without the Investigational New Drug (USA) or Clinical Trial Application (Canada) that are required for other conditions. In the UK, a hospital can prepare FMT and treat its own patients under pharmacy exemption, but a special license is required to send FMT to another hospital. An additional license for an Investigational Medical Product is required to use FMT in a clinical trial. In many other countries, an investigator conducting FMT trials simply needs to submit an application to institutional ethics boards.

An entirely new framework is needed for regulation. We do need regulation of FMT for patient safety. At the same time, we do not want to create barriers in the process to hinder patient access and scientific progress.

Conclusions

FMT is highly effective in treating rCDI and shows promise in treating dysbiosis associated with other GI disorders. Careful donor screening is critical, and a detailed informed consent process is essential. With further understanding of how FMT works, safer and more targeted microbiome therapies will be possible.

References

1. van Nood E, Vrieze A, Nieuwdorp M, et al. Duodenal infusion of donor feces for recurrent Clostridium difficile. N Engl J Med 2013;368:407-15.
2. Kelly CR, Khoruts A, Staley C, et al. Effect of Fecal Microbiota Transplantation on Recurrence in Multiply Recurrent Clostridium difficile Infection: A Randomized Trial. Ann Intern Med 2016;165:609-616.
3. Kao D, Roach B, Silva M, et al. Effect of Oral Capsule- vs Colonoscopy-Delivered Fecal Microbiota Transplantation on Recurrent Clostridium difficile Infection: A Randomized Clinical Trial. Jama 2017;318:1985-1993.
4. Lee CH, Steiner T, Petrof EO, et al. Frozen vs Fresh Fecal Microbiota Transplantation and Clinical Resolution of Diarrhea in Patients With Recurrent Clostridium difficile Infection: A Randomized Clinical Trial. Jama 2016;315:142-9.
5. Cammarota G, Ianiro G, Kelly CR, et al. International consensus conference on stool banking for faecal microbiota transplantation in clinical practice. Gut 2019;68:2111-2121.
6. Jiang ZD, Ajami NJ, Petrosino JF, et al. Randomised clinical trial: faecal microbiota transplantation for recurrent Clostridum difficile infection - fresh, or frozen, or lyophilised microbiota from a small pool of healthy donors delivered by colonoscopy. Aliment Pharmacol Ther 2017;45:899-908.
7. Weingarden AR, Chen C, Bobr A, et al. Microbiota transplantation restores normal fecal bile acid composition in recurrent Clostridium difficile infection. Am J Physiol Gastrointest Liver Physiol 2014;306:G310-9.
8. Seekatz AM, Theriot CM, Rao K, et al. Restoration of short chain fatty acid and bile acid metabolism following fecal microbiota transplantation in patients with recurrent Clostridium difficile infection. Anaerobe 2018.
9. Imdad A, Nicholson MR, Tanner-Smith EE, et al. Fecal transplantation for treatment of inflammatory bowel disease. Cochrane Database Syst Rev 2018;11:Cd012774.

4.3 Fecal microbiota transplantation (FMT), continued.

10. Halkjær SI, Christensen AH, Lo BZS, et al. Faecal microbiota transplantation alters gut microbiota in patients with irritable bowel syndrome: results from a randomised, double-blind placebo-controlled study. Gut 2018;67:2107-2115.