

# A Clinician's Primer on the Role of the Microbiome in Human Health and Disease

Sahil Khanna, MBBS, MS, and Pritish K. Tosh, MD

## CME Activity

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## Abstract

The importance of the commensal microbiota that colonizes the skin, gut, and mucosal surfaces of the human body is being increasingly recognized through a rapidly expanding body of science studying the human microbiome. Although, at first glance, these discoveries may seem esoteric, the clinical implications of the microbiome in human health and disease are becoming clear. As such, it will soon be important for practicing clinicians to have an understanding of the basic concepts of the human microbiome and its relation to human health and disease. In this Concise Review, we provide a brief introduction to clinicians of the concepts underlying this burgeoning scientific field and briefly explore specific disease states for which the potential role of the human microbiome is becoming increasingly evident, including *Clostridium difficile* infection, inflammatory bowel disease, colonization with multidrug-resistant organisms, obesity, allergic diseases, autoimmune diseases, and neuropsychiatric illnesses, and we also discuss current and future roles of microbiome restorative therapies.

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New scientific literature regarding the human microbiome has recently increased, with more than 90% of the nearly 4000 articles indexed by PubMed on the topic being published within the past 5 years. Although the clinical relevance of this field may initially seem esoteric to the practicing clinician, there is growing realization of the role

our commensal microbiota plays in human health and disease. It is likely that in a short time, understanding the basic concepts about the interactions between humans and their microbiome will be as important to clinicians as understanding concepts of genetics or germ theory. This article provides a brief introduction to clinicians of the concepts underlying this

From the Division of Gastroenterology (S.K.) and the Division of Hepatology and Infectious Diseases (P.K.T.), Mayo Clinic, Rochester, MN.

burgeoning scientific field and briefly explores specific disease states for which the potential role of the human microbiome is becoming increasingly evident.

The term *microbiome* refers to the total number of microorganisms and their genetic material and is contrasted from the term *microbiota*, which is the microbial population present in different ecosystems in the body. The average human has 100 trillion microbes in the gut, which is 10 times more than the cells in the human body; hence, the commensal bacteria and fungi that inhabit our bodies vastly outnumber our human cells. The number and variety of bacteria exponentially increase from the proximal to the distal gastrointestinal tract, with the colon harboring most of the gut microbiota. Although it had been largely thought that these microbes are merely tenants on our skin, gut, and mucosal surfaces, it has become increasingly evident that our microbiome is crucial to our health and well-being.<sup>1</sup> The human microbiome has coevolved with humans throughout the millennia, with the development of specific communities of microbes occupying specific anatomical niches within the human body.<sup>2</sup> Human microbial ecology and macroscopic ecology have many parallels that help with the conceptual understanding of the microbiome. Just as one can expect certain kinds of plants and animals on different tropical beaches, one can expect similar microbial ecologic systems in specific anatomical areas that will be common among different people because they are similar, although specific, microecosystems (eg, predominance of Bacteroidetes and Firmicutes in the colon and Firmicutes and Proteobacteria in the mouth).<sup>3,4</sup> The specific balance of microbial diversity within specific anatomical locations will differ among people because of variations, such as in hygiene, social behaviors, and genetics.<sup>5,6</sup> The gut microbiota may differ at different time points at the same anatomical location within the same person owing to environmental changes.<sup>7</sup> Diet plays a major role in defining the composition of the gut microbiota. In addition, metabolites produced by gut bacteria enter the bloodstream by absorption and enterohepatic circulation. Commensal microbiota produces metabolites that may have a positive effect on the host, including anti-inflammatory and antioxidant activity, regulation of gut

barrier function, and production of vitamins and energy sources.<sup>1</sup>

Colonization with normal commensal organisms begins shortly after birth on exposure to vaginal microbiota. Infants continue to be introduced to new flora through routine activities with other humans, including feeding and play, resulting in the establishment of the microbiome on the skin, gut, and mucosal surfaces. Introduction and reintroduction of flora continues throughout life from our routine interactions with each other. The establishment of the gut microbiota starts at birth, reaches its maximum diversity at adolescence, and remains stable until the later stages of life, where the microbiota becomes comparatively less diverse with reduced stability, thus predisposing elderly individuals to conditions associated with decreased diversity, such as *Clostridium difficile* infection (CDI).<sup>8</sup> The 4 predominant bacterial phyla in the gut are Firmicutes, Bacteroidetes, Actinobacteria, and Proteobacteria, followed by archaea, viruses, and fungi.

Rather than simply occupying space on our bodies, our microbiome is essential to several aspects of normal development through interactions with the mucosal immune system. The interaction of our flora with our immune system results in several processes, such as the secretion of secretory IgA and the release of endogenous antimicrobial peptides, among others, that help maintain normal homeostasis of the microbiome.<sup>9</sup> These interactions are also vital to the maturation and maintenance of the mucosal immune system, abnormalities of which have been linked to diseases of anergy and autoimmunity.<sup>9,10</sup> Several aspects of life in modern society, such as antimicrobials, sanitation, vaccination, and dietary changes, have profound and lasting effects on our microbiome. Alterations and imbalance of the gut microbiome have been implicated in gastrointestinal illnesses such as CDI, antibiotic-associated diarrhea, irritable bowel syndrome (IBS), and pathogen colonization (such as vancomycin-resistant enterococcus), and systemic conditions, such as autoimmune and allergic diseases, metabolic derangements (eg, obesity), and neuropsychiatric conditions (eg, autism).<sup>5,7,11-16</sup> Most of the studies that provide information regarding the role of the gut microbiota in disease report alterations of specific community structures in individuals with disease compared with healthy

individuals, thus merely implying association and not proving causation.

The reason for our burgeoning knowledge of the microbiome in the past several years is the acceleration of the technologies that allow for rapid identification and quantification of the highly diverse organisms that comprise the human microbiome, most of which are not culturable with routine microbiologic techniques.<sup>17</sup> High-throughput DNA sequencers are now available that can rapidly and inexpensively sequence specific regions in 16S or 18S ribosomal genes that allow for rapid and inexpensive identification of organisms and their relative abundance in a specimen from which DNA is purified (eg, stool). Because the number of organisms contained in samples (eg, stool samples) is immense, understanding the meaning of the results of studies using this new technology has only been possible through a commensurate acceleration in the bioinformatics needed to interpret the data.<sup>17,18</sup> Similar to the Human Genome Project, the National Institutes of Health supports the Human Microbiome Project, which aims to understand the diversity and relative abundance of our commensal microbiota at different anatomical sites and their role in human health and disease.<sup>17</sup> Although the study of the human microbiome and its role in health and disease states is relatively new, many exciting associations have been discovered that are starting to reveal how important our commensal microbiota is to our health and well-being (Table).<sup>1</sup>

**SELECTED DISORDERS ASSOCIATED WITH MICROBIOME ALTERATIONS**

***Clostridium difficile* Infection**

The most common cause of hospital-acquired diarrhea is CDI, which affects more than 1 million in the United States every year and is the disease for which disruption of the microbiome has most clearly been demonstrated to be causal. Risk factors for CDI include increasing age, antibiotic exposure, hospitalization, presence of severe comorbidities, inflammatory bowel disease (IBD), malignant tumors, and chemotherapy, all of which are associated with decreased gut microbial diversity.<sup>19,20</sup> Systemic antibiotic use is the most widely studied risk factor, which disrupts the normal gut microbiota and leads to an increased predisposition to CDI. The pathophysiology of recurrent CDI involves ongoing disruption of the normal gut microbiota and an inadequate host immune response.<sup>11</sup> Most patients with CDI respond to conventional therapies, such as metronidazole or vancomycin. The risk of recurrent CDI is 20% to 30% in patients with first CDI and increases up to 60% after 3 or more infections.<sup>19</sup> Standard CDI treatment with antibiotics such as metronidazole and vancomycin may further disrupt colonic microbial communities that normally keep expansion of *C difficile* in check. Because *C difficile* spores are resistant to antibiotic therapy for CDI, these germinate to vegetative forms after treatment has been discontinued, leading to recurrent CDI.

**TABLE. Diseases and Conditions With Potential Links to the Human Microbiome**

Disease or condition	Proposed mechanism	Evidence	Possible therapies available to alter microbiota
CDI	Reduced microbial diversity	Animal and human studies	FMT for treatment of recurrent CDI and questionable use of probiotics for prevention of CDI
IBS	Reduced microbial diversity and decreased Bacteroidetes	Animal and human studies	Probiotics for treatment of IBS
Inflammatory bowel disease	Reduced microbial diversity	Human studies	Probiotics (VSL #3) for treatment of pouchitis, trials of FMT ongoing
Obesity and metabolic derangements	Reversed Firmicutes to Bacteroides ratio	Animal and human studies	Trials of FMT ongoing
Allergic disorders	Reduced microbial diversity	Animal and human studies	Studies of probiotics ongoing
MDRO colonization	Reduced microbial diversity	Human studies	Studies of probiotics ongoing
Neuropsychiatric illnesses	Disruption of intestinal barrier	Animal and human studies	None

CDI = *Clostridium difficile* infection; FMT = fecal microbiota transplantation; IBS = irritable bowel syndrome; MDRO = multidrug-resistant organism.

For patients with multiple CDI recurrences, fecal microbiota transplantation (FMT) is now accepted as a safe and effective (with >90% cure rates on the basis of data from a randomized controlled trial and multiple case series) alternative to standard antibiotic therapy because of the ability to restore the colonic flora via infusion of a liquid suspension of intestinal microorganisms from the stool of a healthy donor.<sup>11,19,21</sup> Considerations for FMT include appropriate donor screening (for transmissible infectious and other diseases) and selection, standardization of stool preparation, insurance reimbursement for donor testing, and long-term safety, efficacy, and adverse effects from FMT in patients with CDI. Although FMT is not approved by the Food and Drug Administration, it is permitted by the Food and Drug Administration after discussion of risks and benefits with patients. Currently, FMT is being performed as clinical practice and under research protocols for CDI at many academic centers all over the world.

### Irritable Bowel Syndrome

The most commonly diagnosed gastrointestinal condition is IBS, with an estimated prevalence of 10% to 15%. It is characterized by chronic abdominal pain and altered bowel habits (diarrhea or constipation) in the absence of a structural or organic cause. The pathophysiology of IBS is postulated to include alterations in the gut motility, enhanced visceral hypersensitivity, postinfectious states, food sensitivity, and, more recently, a possible role of altered intestinal microbiota.<sup>22</sup> Data have suggested that gut microbiota in individuals with IBS differs (decreased diversity and decreased Bacteroidetes) from healthy controls and may also vary with the predominant IBS symptom of constipation or diarrhea.<sup>23,24</sup>

The mainstay of treatment for IBS is on the basis of the predominant symptoms (antidiarrheals, antispasmodics, antidepressants, and dietary alterations, such as low carbohydrate and fermentable sugars); however, some evidence supports treatments guided at alterations of gut microbiome, such as antibiotics, probiotics, and case series of FMT.<sup>25-27</sup> In randomized controlled trials, rifaximin led to symptomatic improvement in global IBS symptoms and bloating, and several short-term controlled trials of probiotics in IBS have found a small benefit,

but due to modest benefit antibiotics or probiotics are not routinely recommended for treatment of IBS.<sup>26,27</sup> Published case reports of FMT for treatment of IBS have reported improvement, but there may be publication bias because of negative reports not being published.<sup>25</sup> There is heterogeneity in the individual microbiota variation with IBS phenotypes, and larger studies are needed to phenotypically characterize these patients to more clearly identify the contribution of gut microbiota alterations in patients with IBS.<sup>22</sup>

### Inflammatory Bowel Disease

The 2 major subtypes of IBD include ulcerative colitis and Crohn disease, both of which have an inheritable genetic component. Genome-wide studies suggest that factors other than genetic mutations, such as altered microbes, are a major environmental factor for development of IBD because the gut microbiota has a major role in the barrier function and immune regulation of the gut. Mouse models of IBD do not develop experimental colitis in a germ-free environment and require the presence of intestinal microbes for the development of colitis, suggesting a role of the gut microbiota in the pathogenesis of IBD. In the presence of a genetic predisposition to develop IBD, abnormal interactions between the altered gut microbiota and the mucosal immune system may lead to chronic intestinal inflammation.<sup>28</sup>

Studies have found that the gut microbiota has reduced species diversity, lower temporal stability, and structural disruption of the secreted mucous layer in patients with ulcerative colitis and Crohn disease, suggesting an association.<sup>12,29</sup> However, it remains unclear whether the compositional and architectural changes in the microbiota are the cause or a result of inflammation and diarrhea. Clinically, fecal diversion often leads to improvement of downstream inflammation in Crohn disease. There is controversial evidence outlining the effect of FMT for patients with IBD; therefore, FMT is currently not an option to manage IBD.<sup>30</sup> However, randomized trials are ongoing, and currently FMT for IBD must be performed only in research settings. Probiotics have been studied for IBD, and the evidence is insufficient to recommend use of probiotics except for the probiotic VSL #3 in chronic pouchitis.<sup>31</sup>

### Colonization With Multidrug-Resistant Organisms

With increasing understanding of the gut microbiota, maintaining a healthy gut microbiota has been postulated as a key factor to prevent colonization and infection with multidrug-resistant organisms (MDROs) (Figure).<sup>32</sup> Competition for space and resources and the complex immunologic and biochemical interactions between an intact gut microbiota and the host may prevent colonization and infection with MDROs in the healthy state. Current antimicrobials used to treat infections cause tremendous collateral damage to the human gut microbiota, which may be a driving force behind the introduction and proliferation of MDROs, such as vancomycin-resistant enterococcus, extended-spectrum  $\beta$ -lactamase producers, and *Klebsiella pneumoniae* carbapenemase producers.<sup>32</sup> Ongoing research is needed to better define host gut microbiota in patients with MDROs and delineate potentially beneficial methods to normalize the composition by methods such as targeted antibiotics or microbiota restorative therapy to potentially eradicate MDROs.

### Obesity and Metabolic Derangements

Obesity has recently been categorized as a disease by the American Medical Association and has reached an epidemic state in the United States. Obesity is associated with hypertension, hyperlipidemia, fatty liver, hypertension, diabetes mellitus, heart disease, and a state of systemic low-grade and chronic inflammation. The 100 trillion bacterial residents in the human gut comprise approximately 4 lb of fecal matter. These bacteria maintain a high population density by deriving nutrients from food sources and the intestinal epithelial lining. For example, butyrate, which is produced by bacterial fermentation of dietary fibers, may serve as an energy source for the intestinal epithelial lining and increase satiety.<sup>13,14</sup> Mouse studies have found that obese mice have a 50% reduction in the abundance of Bacteroidetes and a proportional increase in Firmicutes compared with lean mice.<sup>33</sup> A similar ratio has been seen in humans, and it has been observed that the ratio of Firmicutes to Bacteroidetes decreases with weight loss and after bariatric surgery.<sup>34</sup> Fecal transplants from obese mice transplanted into lean germ-free mice have been found to induce obesity.<sup>35</sup> The composition of the microbiota influences

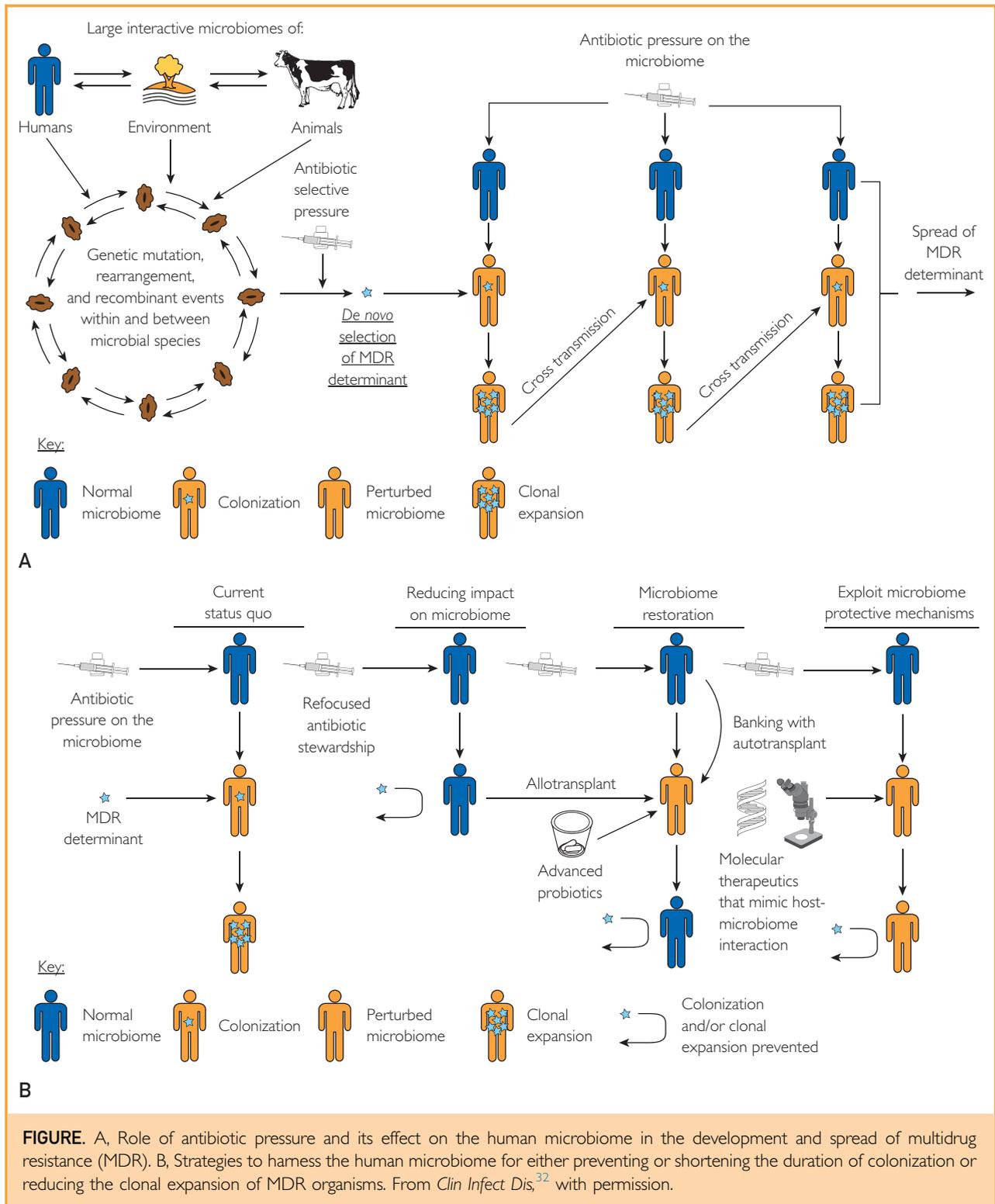
the host through variable efficiency of energy extraction from the diet, depending on relative composition of the bacterial species. A recent study found an improvement in insulin resistance in obese individuals who received fecal transplants from lean individuals compared with those who received autofecal transplants.<sup>36</sup> The changes seen in the gut microbiome in relation to body mass index is a strong correlation, but the causality of changes has not been established as yet. The interaction among human genetics, diet, and the human gut microbiota still remains to be completely defined and may lead to development of guidelines to modify the gut microbiota by diet, prebiotics, or advanced probiotics with a potential to improve metabolic syndrome or obesity.

### Allergic Diseases

In the past few decades, there has been a tremendous increase in the prevalence of allergic diseases, such as asthma, eczema, and food allergies. The hygiene hypothesis has suggested that microbial exposures during childhood are crucial to the development of the immune system, and alterations in the development of the immune system predispose patients to loss of self-tolerance.<sup>15</sup> Recent evidence has suggested that the changes in the intestinal microbiota play a significant role in influencing the immunologic events that could lead to the development of allergic diseases.<sup>15</sup> Altering microbial colonization and exposures during the perinatal period and early childhood, especially with recurrent antibiotic exposure, may promote dysregulated immune responses, leading to allergic and atopic disorders.<sup>15</sup> However, despite strong evidence supporting a role for altered gut microbiota in atopic diseases, treating these disorders using probiotics has been unsuccessful. This may be related to choice or efficacy of the probiotic strains selected, variability in the individuals, or the fact that some of the immune dysregulation caused by altered microbiota may not be completely reversible.<sup>37</sup>

### Neuropsychiatric Illnesses

Dysregulated cross-talk between the brain and the gut immune system may be an important contributor to the pathogenesis of several conditions, including schizophrenia, mood disorders, obsessive-compulsive disorder, autism, attention-deficit/hyperactivity disorder, anorexia nervosa, narcolepsy, and chronic fatigue



syndrome.<sup>16</sup> In genetically susceptible individuals, altered gut microbiota may lead to disruption of the blood brain barrier and generation of brain-reactive autoantibodies, and under inflammatory conditions there may be a disruption of the blood brain barrier that may facilitate transport and then binding of autoantibodies to cross-reactive epitopes, which may contribute to the development of these cognitive and behavioral disturbances.<sup>16</sup> Commensal organisms in the gut are essential for proper brain development and function, and studies have found that specific probiotics or bacteria may modify behavioral abnormalities.<sup>16</sup> Changes in the microbiome may alter the central nervous system via disruption of the integrity of the epithelial barrier of the intestine by the entry of bacterial proteins (eg, p-cresol or 4-methylphenol), with neuroactive properties into the circulation.<sup>16</sup> However, to date it is not known whether there are approaches to modify the gut microbiota to effectively treat these disorders.<sup>16</sup>

### Microbiota Restorative Therapies: The Biotics

Many, largely over-the-counter (OTC), products are available that attempt to modulate the gut microbiome, including prebiotics (compounds that may encourage the growth of one gut species over another), probiotics (oral or rectal intake of biologic species), and synbiotics (combinations of prebiotics and probiotics intended to work synergistically). This has become an expanding industry, with an estimated market in the United States of more than \$1 billion annually.<sup>31</sup> Many OTC probiotic products include live cultures of a microorganism, such as *Lactobacillus acidophilus*, *Saccharomyces boulardii*, and *Bifidobacterium*; however, other products marketed as probiotics are in the form of foods such as yogurts and cultured drinks.<sup>31</sup> Some clinical trial data support the use of certain probiotics for conditions such as CDI, viral diarrhea, antibiotic-associated diarrhea, and traveler's diarrhea; however, prebiotics, probiotics, and synbiotics are often used for purposes such as enhancement of digestion or treatment of chronic fatigue symptoms for which there is little or no supporting clinical data.<sup>31</sup> Although isolated case reports of bloodstream infections and intra-abdominal abscesses have been reported mostly in patients with underlying immunologic or structural abnormalities, the use of probiotics

has generally been safe.<sup>31</sup> Despite substantial enthusiasm regarding the use of probiotics for the treatment of conditions that may stem from disruptions in the microbiome, the use of currently available probiotics alone for the treatment of these conditions has not proven to be curative. This is likely, in part, due to the disparity between the complexity and diversity of the gut microbial ecologic system (mostly unculturable anaerobic microbiota) and the single organism often contained in OTC probiotics. Often, FMT is able to restore the complexity and diversity of the gut flora and has been successful in treating CDI and being investigated for use in other conditions.<sup>11,21</sup> Ideally, advanced probiotics of the future would be able to restore normal colonic diversity without the need for FMT.

### CONCLUSION

In summary, the gut microbiota is essential to maintaining health and may be altered in disease. The composition and function of a healthy gut microbiota remain to be clearly defined. Several disease states have been correlated with alterations of the gut microbiota, although it is not clear whether these alterations are a cause or consequence. The key questions pertaining to the gut microbiota are whether alterations in the microbiota are causative or merely associations with diseases and whether therapeutically manipulating the gut microbiota by dietary changes, microbiota restorative therapies, or immune modulation would alter disease course.

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**Abbreviations and Acronyms:** CDI = *Clostridium difficile* infection; FMT = fecal microbiota transplantation; IBD = inflammatory bowel disease; IBS = irritable bowel syndrome; OTC = over-the-counter; MDROs = multidrug-resistant organisms

**Correspondence:** Address to Pritish K. Tosh, MD, Division of Infectious Diseases, Mayo Clinic, Rochester, MN 55905 (tosh.pritish@mayo.edu).

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