

# The Microbiome in Rheumatic Diseases and Infection

Gaafar Ragab  
T. Prescott Atkinson  
Matthew L. Stoll  
*Editors*

 Springer

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*It has long been an axiom of mine that the little things  
are infinitely the most important*

*—Sherlock Holmes in A Case of Identity  
by Sir Arthur Conan Doyle.*

*This book is dedicated to our wives: Samia, Miriam,  
and Janice.*

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## Foreword

It is fitting to now have a book dedicated entirely to the role of the microbiome in our rheumatic diseases. After all, the study of the infectious origins of rheumatoid arthritis alone is an exercise in exploration of the modern bestiary. From 30,000 feet I would like to draw attention to observations that may help to open our minds on this topic before entering this valuable new addition to the literature which bridges the fields of rheumatic and immunologic diseases and microbiology.

First, while I am genuinely excited at the exploration of the interface of rheumatic disease etiology, pathogenesis, and natural history and the human microbiome, I am more basically left in wonder by how our microbiome shapes our relationships not just with diseases but with our sum total of experiences with the natural world. Recognize that we are not humans at all but exist as superorganisms or holobionts who have been imprinted with a remarkable spectrum of microbial entities whose own interests may not coincide with ours at any given time. Furthermore and even more remarkable is that our DNA is about 8–10% of viral origin (i.e., endogenous retroviral elements) that has created a host-parasite co-evolutionary dynamic affecting everything from our integrated host defenses to our behavior. Based on this remarkable fact alone it is imperative that we increasingly dedicate our precious resources to furthering our understanding of these relationships and how they contribute to enhancing health and causing disease.

Second, I would like to remind us that the study of the microbiome and its relationship to health and diseases is a long road and that reductionist aspirations to find a microbial culprit that causes a given disease or to therapeutically manipulate the microbiome through microbial supplements or dietary change are likely to be unrewarding, at least for now. Study of the microbiome in many ways, including the massive global efforts to categorize it such as the Human Microbiome Project and the Earth Microbiome Project, remind me in many ways of the excitement and effort poured into the Human Genome Project which in the end taught us relatively little about specific human diseases but opened up a Pandora's box of ever new questions to be addressed. While we hope that some clarion concepts may arise from such reductionist approaches to the microbiome, we may find ourselves in the same situation, facing an ever increasing and complex set of questions to address if we are to move forward. To do so I urge us all to think expansively and consider how our microbiome interacts with other networks such as our food supply, our environment, and our society.

So with these humbling caveats which hopefully remind us of our small vantage point in our complex world I welcome you to *The Microbiome in Rheumatic Diseases and Infection*.

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**Gaafar Ragab** graduated from the Faculty of Medicine, Cairo University, 1976, where he got his degrees in Internal Medicine: MSc, 1980, and PhD, 1985. He served his faculty as a Resident, Assistant Lecturer (1980), Lecturer (1985), Assistant Professor (1990), and Professor (1995) till now. He spent a sabbatical year 1989 at the UAB, USA, in the Department of Clinical Immunology and Rheumatology. He headed the Clinical Immunology and Rheumatology Unit affiliated to the Internal Medicine Department (2010–2015). He was Chief of the Internal Medicine Department's Research Committee (1995–2013). He is Fellow of the American College of Rheumatology (ACR) since 1989. He is a Co-founder of the Egyptian Society of Immunology and Rheumatology (EGYSIR) which he headed as its President from 2010 to 2013. He was chosen as President of the Egyptian League Against Rheumatism (ELAR) annual meeting in Alexandria, 2013. He is a member of the Advisory Board of the Egyptian Society of Internal Medicine (ESIM) and its journal and served as President of ESIM annual meeting, Cairo, 2014. He is the associate editor of the *Journal of Advanced Research* (JARE), the interdisciplinary publication of Cairo University, in charge of its medical branch. He won the prize of honor, Cairo University, for the great efforts in international publications, for the year 2008. He is a member of the Egyptian National Committee for the management of Hepatitis C Virus (the extrahepatic manifestation) as well as the International Study Group of Extrahepatic Manifestations Related to Hepatitis C Virus infection (ISGEHCV). He is also a member of the Geographical Variation in Rheumatoid Arthritis Group (GEO RA).



**T. Prescott Atkinson** is a native of Alabama, USA, born and raised in Montgomery. He attended Tulane University (1971–1975) in New Orleans, Louisiana, and then completed 6 years of active duty in the U.S. Navy. After his military tour of duty, he entered the Medical Scientist Training Program (MD-PhD) at Emory University (1981–1987). It was during his PhD work at Emory University that he became interested in immunologic cell signaling, and he carried this area

of interest in immunology into Fellowship training at the National Institutes of Health as an NIAID Clinical Fellow in Allergy/Immunology (1987–1992). After moving to the University of Alabama at Birmingham in 1992 as an Assistant Professor of Pediatrics, he began seeing pediatric rheumatology and primary immunodeficiency patients in clinic, and benefited immensely from weekly review conferences/patient discussions with Dr. Max Cooper, one of the giants in the field of immunology. This is an area in which he still maintains an active clinical interest, and over the years, principally in collaboration with groups at the NIH, he has continued to pursue studies on the molecular etiology and clinical characteristics of this extraordinarily diverse group of disorders. He has also become interested in the role of chronic infection, particularly with mycoplasmas, in chronic diseases such as asthma and arthritis, particularly in patients with primary immunodeficiencies and rheumatic disorders. He is a former member and Chair of the American Board of Allergy and Immunology. Currently he is Professor and Director of the Division of Pediatric Allergy, Asthma, and Immunology at UAB.



**Matthew L. Stoll** is a pediatric rheumatologist who earned his MD and PhD at Upstate Medical University (Syracuse, NY) in 2001, followed by completion of residency in pediatrics at the Long Island Jewish Medical Center in 2004 and fellowship in pediatric rheumatology at the Children's Hospital Boston in 2007. At the completion of his fellowship, he took a staff position at the University of Texas at Southwestern Medical Center (Dallas, TX), where he earned an

MSCS degree. In 2011, he moved to the University of Alabama at Birmingham, where he remains. During his fellowship, Dr. Stoll developed an interest in the clinical epidemiology of spondyloarthritis, publishing on age-based subgroups of juvenile psoriatic arthritis as well as risk factors for sacroiliitis in patients with pediatric spondyloarthritis. He subsequently turned his attention to the links between spondyloarthritis and inflammatory bowel disease and reported on the use of fecal calprotectin as well as intestinal MRI to identify subclinical intestinal inflammation in children with spondyloarthritis. His current work focuses on the role of the intestinal microbiota in the pathogenesis of pediatric spondyloarthritis.

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**Part I**

**Introductory Chapters**



# The Microbiome: Past, Present, and Future

# 1

Matthew L. Stoll

## Abbreviations

AS	Ankylosing spondylitis
EEN	Exclusive enteral nutrition
IBD	Inflammatory bowel disease
JIA	Juvenile idiopathic arthritis
RA	Rheumatoid arthritis

## Past: Surprising Insights into Today's Microbial World

All disease starts in the gut.—Attributed to Hippocrates

In the 1670s, Antony van Leeuwenhoek was the first to describe the presence of bacteria, which he described as “animalcules of the most minute size which moved themselves about very energetically [1].” Very little progress was made toward identifying or characterizing bacteria over the next two centuries. Infectious agents had not, it appears, captured the attention of the scientific community until Louis Pasteur promoted the concept that germs can cause transmissible disease, and Pasteur

as well as Robert Koch further contributed to the field by developing techniques to culture bacteria [2]. As reviewed in 1911 [1], in the 1870s, two independent groups detected the presence of bacteria in stool. However, much of the work at the time, quite understandably, was focused on isolation of specific organisms associated with devastating diseases. Along those lines, there were some major discoveries at the time, including discovery of the bacteria causing anthrax in the blood of a dead animal accompanied by the demonstration that the disease could be transmitted through injection of the blood into a healthy animal as well as isolation and identification of the bacteria causing such diseases as tuberculosis, bacterial dysentery, and cholera [1]. Of note, the investigator who discovered both *Mycobacterium tuberculosis* in 1882 and *Vibrio cholerae* in 1884, Robert Koch, is still known today for his work proving pathogenicity of these bacteria.

Interest in the intestinal microbiota as a whole did not emerge until early in the twentieth century. Elie Metchnikoff had a rather dismal view of the microbiota, fearing that it released toxins into the systemic circulation that produced senility, and he therefore advocated altering the colonic microbiota [3]. An extreme method of doing so, which gained some attraction in the early twentieth century, was colectomy. There were some adherents to this belief, including Dr. Arbutnot Lane, who performed colectomy or colonic bypass for a variety of

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indications [4]. By the 1920s, this procedure had fallen out of favor [3].

A more nuanced view of the intestinal microbiota was offered by Arthur Kendall, who hypothesized that they were typically benign, unless the host is colonized with specific pathogenic agents [1]. That the intestinal microbiota was essential for the health of the host was initially demonstrated in 1915, through studies on germ-free chicks, which showed poor development of the germ-free animals starting at 10 days of life [5]. These observations resulted in the conclusion that “man has a bacterial population in his intestinal tract; that under normal conditions the organisms in the intestinal tract are fairly characteristic and constant; normally they are harmless; [and] *they may be protective* [5].”

In addition to work in germ-free animals, several further lines of current research into the microbiota had their start 100 years ago. One of them is the functional capacity of intestinal bacteria, which today is studied through such tools as shotgun sequencing of microbial DNA and mass spectroscopy of fecal and plasma metabolites. Ford initially noted that bacteria differ in their ability to metabolize carbohydrates and proteins, characterizing bacteria into two categories: fermenters (carbohydrates metabolizers) and putrifiers (protein metabolizers) [6]. Kendall extended these findings, observing that “Food largely determines the type of intestinal bacteria [1].” Specifically, diets rich in carbohydrates resulted in the generation of bacteria with increased capacity to metabolize carbohydrates. Today, it is well recognized that fiber-rich diets result in increased abundance of bacteria capable of metabolizing complex carbohydrates [7]. While carbohydrate and protein metabolism were the focus of attention in the first two decades of the twentieth century, by mid-century, the microbial effects on multiple other endogenous substances were studied, including B-complex vitamins [8–10], vitamin C [11], and cholesterol [12].

Another area of active interest today that had its roots 100 years ago is interest in treating disease through alterations in the intestinal microbiota. While today’s efforts, as will be seen throughout this textbook, focus on the treatment

of chronic inflammatory diseases, interest in the pre-antibiotic era was in the management of infectious diseases. As discussed above, colectomy was an extreme method of altering the intestinal microbiota, but not the only one. Diet has long been recognized as a very effective means of doing so, beginning with observations from 1911 that bottle-fed and breast-fed infants had substantially different microbial populations, with these studies even showing increased “homogeneity” of the intestinal microbiota in bottle-fed infants [1]. These observations are a precursor to recent findings showing decreased alpha diversity in bottle-fed compared to nursed infants [13]. Torrey as well noted that diet strongly influenced the contents of the microbiota, writing “It has been my experience that the intestinal flora of dogs reacts very promptly and with great uniformity to changes in diet [14].” Kendall proposed using simple sugars to alter the microbiota as a therapeutic tool for bacterial dysentery, thus in effect introducing the first instance of a therapeutic prebiotic [1]. Lane followed therapeutic colectomies in the first decades of the twentieth century with introduction of pure cultures of bacteria, first *Lactobacillus bulgaricus* and later *Lactobacillus acidophilus*, an early use of probiotics [3]. In perhaps the first published fecal microbial transplant, Dalton transplanted *Escherichia coli* from a healthy subject to a child undergoing antibiotic therapy for meningitis, reporting that rectal but not oral administration of the organism resulted in successful uptake and may have contributed to resolution of the illness [15]. In 1955, Winkelstein evaluated *Lactobacillus acidophilus* as a therapeutic agent in 53 subjects with a variety of intestinal disorders, including ulcerative colitis, reporting mixed results [16]. For the most part, however, interest in probiotics remained low until the 1990s [3].

Loss of interest in probiotic therapy as a tool to alter the microbiota may have been due to the development of antibiotics, with penicillin introduced in 1928 and many others to follow. Improved public health measures in developed nations, including vaccinations and improved hygiene, likely also dampened enthusiasm in research into microbial-based therapy of intestinal

infections. In any event, the widespread use of antibiotics spurred interest in the 1940s and 1950s on the effect of these therapies on the contents of the intestinal microbiota [17–20] and subsequently on the development of antibiotic resistance [21]. Another line of research in that era that pertained to antibiotics, which at the time was largely of interest to the agricultural field, were the effects of antibiotic therapy on the growth of livestock. Several studies demonstrated that young animals fed antibiotics demonstrated increased growth [22–24]. Observations that these growth-promoting effects of antibiotics did not occur in germ-free animals [25] and were associated with increased efficiency of absorption of dietary fatty acids [26] resulted in the conclusion that changes in the fecal microbiota mediated the increased weight gain of young animals treated with antibiotics [26]. Although this practice has fallen in disfavor due to concerns of transmission of antibiotic-resistant bacterial pathogens to humans, interest in the effects of antibiotics on growth remains, with a recent study showing that early exposure to antibiotics may be associated with an increased risk of childhood obesity [27].

One final theme that emerged in the 1950s and is germane to this textbook is the association of the intestinal microbiota with autoimmune diseases, including those not intrinsic to the gastrointestinal tract. Perhaps the first such study was published by Seneca, who reported increased total and coliform bacteria in the feces of 15 patients with UC as compared to four healthy controls [28]. Studies in the 1950s evaluated the intestinal microbiota in pediatric celiac disease [29] and acne [30]. Subsequent early studies on the intestinal microbiota were published in Crohn disease in 1969 [31], rheumatoid arthritis (RA) in 1966 [32], and ankylosing spondylitis (AS) in 1978 [33].

Ultimately, all of these efforts were limited by technology. For 100 years following the resurgence of interest in the intestinal microbiota, the only tool available to characterize them was culture, which we know today to be a highly inefficient means to characterize bacteria. It is often cited that only 20% of intestinal bacteria can be

cultured [34]. Although this number may be higher [35], many of these bacteria require specialized media, and anaerobic culture is also technically demanding. In 1977, Carl Woese introduced the concept of identifying bacteria according to their ribosomal 16S DNA sequence [36], and 10 years later he published an immense database of bacterial 16S sequences [37]. This permitted use of DNA probes to characterize bacterial communities, and this technology was used in studies of RA [38] and AS [39] to name but two. However, the real explosion in microbial DNA technology had yet to come.

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### Present: “Democratization of Metagenomics”

The intestinal tract is a wonderfully perfect incubator and culture medium combined... It must be evident that the direction that this flora takes will not be without influence upon the host.—Arthur Kendall (1911)

The last 10 years has witnessed an explosion of research into the microbiota. A PubMed search of microbiome or microbiota identified nearly 40,000 publications, the vast majority of which are under 10–15 years old. This research has been enabled by advances not only in sequencing technology but primarily in computing power; indeed, a typical smartphone contains more than 100,000 times the computing power of those that launched the lunar mission in 1969. More recently, even the initial sequencing of the Human Genome Project costs over \$3 billion and took approximately 13 years, whereas today, the estimated cost of whole human exome sequencing is under \$1000 <http://www.genome.gov/sequencingcosts/> (accessed December 18, 2017). Due to the lower costs, investigators around the world are able to contribute to the field, a capacity that Jeff Gordon dubbed the “democratization of metagenomics [40].” These efforts around the world have been tremendously supported by massive centralized efforts to catalog the microbiota: the Human Microbiome Project in the United States [41] and Euro-HIT in Europe [42]. Thanks in no small part to these efforts, reference databases contain over

1.4 million bacteria and 53 thousand archaea [43] as of the end of 2016.

Much of the human work involving the microbiome consists of identifying differences in the microbiota between patient groups, e.g., those with versus without a particular disease. Such work is open to criticism that these differences are associative, but do not necessarily reflect a causal relationship. That is, the inflammatory milieu associated with a particular disease, or even its treatments, may result in alterations in the microbiota that are challenging to control for using comparison groups of healthy individuals. However, important work in animals and even in humans to some extent has shown the power of the microbiota to shape the disease, as well as the therapeutic potential of alterations of the microbiota.

Multiple animal models of inflammatory disease are attenuated or in some cases accelerated when the animals are raised in a germ-free setting, either in a true gnotobiotic facility or through treatment with broad-spectrum antibiotics. These include models of RA [44], ulcerative colitis [45], and chronic noninfectious osteomyelitis [46]. In each of these models, disease was highly attenuated in the germ-free state, and, furthermore, Koch's postulates of disease causation were partially established by recurrence of the disease when the microbiota were reintroduced into the animals.

A striking example of mediation of disease through the microbiota is the transfer of the obesity phenotype. Turnbaugh et al. studied mice that were genetically programmed to develop obesity based upon mutations in the gene coding for the satiety signal leptin [47]. Obese mice had increased *Firmicutes* in their intestines, findings typical in the obese state. Impressively, transfer of the fecal microbiota to germ-free mice resulted in increased weight gain among mice that received microbiota from obese as compared to lean mice. There were no differences in chow consumption, so this difference reflected increased energy harvest.

Another example is the HLA-B27 transgenic rat model of spondyloarthritis. Typically, transgenic rats develop a spontaneous arthritis, orchitis, and colitis.

When raised in a sterile environment, the rats are protected against arthritis and colitis [48]; however, disease recurs when the animals are exposed to a cocktail of bacteria that includes *Bacteroides vulgatus* [49].

Human studies as well demonstrate that the microbiota can impact inflammatory diseases. One interesting illustration of this came from research in infants at risk for type I diabetes mellitus based upon HLA types [50]. The investigators obtained serial fecal specimens from 33 at-risk children from birth through age 3 years, finding that changes in the contents of the fecal microbiota preceded development of clinical disease.

Similarly, a study of adults with newly diagnosed RA showed an expansion of a single organism, *Prevotella copri*, in 75% of newly diagnosed subjects, that was not seen in healthy controls or established patients [51]. The pathogenic nature of this species was further demonstrated by oral gavage of mice, which resulted in colitis.

Finally, the impact of the microbiota on human disease is illustrated by therapeutic responses to treatment, possibilities that are still in their infancy. While antibiotic [52] and probiotic [53] therapy have long been a mainstay of treatment of inflammatory bowel disease, there has been increasing interest in the potential role of fecal microbial transplantation [54]. Additionally, it is clear that dietary manipulation through the use of exclusive enteral nutrition (EEN) can induce remission of inflammatory bowel disease (IBD) as effectively as can corticosteroids [55, 56], and EEN has also been reported to be beneficial in children with juvenile idiopathic arthritis [57]. Although dietary changes can induce rapid shifts in the microbiome [58], it is not clear whether the beneficial effects of dietary changes are mediated through the microbiome or some other mechanism. It remains to be seen whether microbial manipulation will have similar effects in other diseases.

It is not at all surprising that alterations in the microbiota can impact inflammatory diseases. The microbiota is required for normal development of the immune system [59], and the intestinal microbiota in particular represents the largest mass of microbial antigen and adjuvant that is encountered in life, thus setting the stage

for marked effects on systemic and mucosal immune systems [60]. Indeed, antibodies directed against commensal microbial components are present and potentially pathogenic in a variety of autoimmune diseases, including IBD [61], spondyloarthritis [62], and RA [63].

Finally, it bears mentioning that certain microbiota may also be beneficial. Not only are certain bacteria generally considered protective (e.g., *Faecalibacterium prausnitzii* in IBD (Chap. 19)), but there is a body of literature that an entire class of organisms, helminth parasites, may also be protective against allergic or autoimmune diseases. The data in mice were summarized in a recent review [64]. Evidence that parasitic infection may be protective against allergy or autoimmunity is as follows: (a) A meta-analysis determined that current infection with an intestinal

parasite was associated with reduced risk of allergic sensitization [65]; (b) worldwide rates of multiple sclerosis and parasitic infestation show an inverse correlation [66]; and (c) in an area endemic for filarial parasites, patients with RA were significantly less likely to be infected as compared to healthy controls [67]; an observational study of multiple sclerosis patients demonstrated that helminth infection was associated with reduced disease progression [68]. It does bear mention, however, that some studies have shown contradictory data with respect to helminth infection and atopic diseases [69–71], and consequently not all investigators have been convinced by the epidemiologic data [72]. Additionally, interventional studies of live parasites in a variety of human autoimmune disorders have generally shown mixed results (Table 1.1).

**Table 1.1** Therapeutic trials of parasitic worms

Study	Patient population	Study design	Parasite	Outcome
<b>Allergic rhinitis</b>				
[79]	100 adults	RCT	<i>Trichuris suis</i>	No improvement in symptoms
[80]	100 adults	RCT	<i>Trichuris suis</i>	No changes in allergic reactivity
<b>Asthma</b>				
[81]	30 adults	RCT	<i>Necator americanus</i> larvae	No improvement in airway hyperreactivity
[82]	32 adults	RCT	<i>Necator americanus</i> larvae	No improvement in airway hyperreactivity
<b>Inflammatory bowel disease</b>				
[83]	4 adults with CD and 3 with UC	OL, uncontrolled	<i>Trichuris suis</i>	6/7 achieved remission for at least part of the study period
[84]	29 adults with CD	OL, uncontrolled	<i>Trichuris suis</i>	At week 24, 21/29 (72%) responded; 23/29 (79%) met criteria for remission
[85]	54 adults with UC	RCT	<i>Trichuris suis</i>	Favorable response seen in 13/30 (43%) in the treatment group versus 4/24 (15%) controls ( $p = 0.04$ ). Remission occurred in $\leq 10\%$ in both groups
[86]	36 adults with CD	RCT	<i>Trichuris suis</i>	Improvements in symptoms seen in placebo and treatment groups; no comparisons performed
<b>Multiple sclerosis</b>				
[87]	5 treatment-naïve adults	OL, uncontrolled	<i>Trichuris suis</i>	Decrease in number of new MRI lesions from 6.6 to 2; no change in self-reported symptoms
[88]	10 adults	OL, uncontrolled	<i>Trichuris suis</i>	Increase in number of new MRI lesions from 6 to 21
[89]	16 treatment-naïve adults	OL, uncontrolled	<i>Trichuris suis</i>	Nonsignificant improvement in MRI lesions; self-reported improvement in symptoms in 12/16

CD Crohn disease, MRI magnetic resonance imaging, OL open-label, RCT randomized controlled trial, UC ulcerative colitis

It is of particular interest that we have come full circle in our understanding that some of the chronic rheumatic diseases may have microbial causes. Over a century ago, C. Fred Bailey proposed that RA was likely caused by toxins elaborated by microorganisms, which potentially resided in the joints, nasopharynx, or gastrointestinal tract [73]. Sulfasalazine was developed as a therapeutic agent on the basis of this assumption that RA is an infectious disease [74]. Indeed, as discussed in the RA chapter (Chap. 15), there have been multiple successful trials of antibiotics in RA, yet by the late twentieth century, the notion that this was an infectious illness was abandoned, and the effectiveness of antibiotics was attributed to intrinsic anti-inflammatory effects of these agents [75]. Yet now, as shall be discussed as well in the RA chapter (Chap. 15), there is substantial evidence that specific microbes and their associated inflammatory properties underlie the disease.

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### Future: Microbiota-Based Therapeutics or Prevention

A lack of knowledge of the normal intestinal bacteria and their relations will be a serious handicap in recognizing the abnormal bacteria and their relations... Arthur Kendall (1911)

Much work lies ahead to understand not only the contributory role of the microbiota to the disease but also the extent to which microbial manipulation may have therapeutic potential. As with any medication, this will require well-designed randomized studies to assess safety and efficacy. Many rheumatologists are familiar with the concept of a “window of opportunity” to treat an inflammatory disease. We are also familiar with the idea that the disease process begins long before the first symptom emerges, as illustrated by lupus-associated antibodies being formed years before the clinical onset of disease [76]. For diseases mediated by the microbiota, the window may be long before even the first disease manifestation. We will learn in the juvenile idiopathic arthritis (JIA) chapter (Chap. 17) of evidence that elevated fecal *Bacteroides* in JIA may reflect not intrinsic pathogenicity of this genus but altered

immune development on account of it. We are also learning that early childhood events affecting the gut microbiota may influence the risk not only of pediatric autoimmune disease but possibly even adult disease as well. Gordon proposed the concept of microbial prevention, such as administering probiotics to infants immediately after birth, or even to their mothers just before delivery [40]. Probiotic studies involving infants have shown benefit in reducing the risk of type I diabetes [77] and atopy [78]. Thus, the future of microbiota-based therapeutics may prove to be as much of a public health measure as therapeutic measures for individual diseases.

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# Methods for Microbiota Analysis: Sample Collection and Laboratory Methods

# 2

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## Abbreviations

CD	<i>Clostridium difficile</i>
CD	Crohn's disease
CDI	<i>Clostridium difficile</i> infection
DGGE	Denaturing gradient gel electrophoresis
FACS	Flow cytometry (FCM) fluorescence-activated cell sorting (FACS)
FISH	Fluorescence in situ hybridization
GC-MS	Gas chromatography-mass spectrometry
MAR	Microautoradiography
OTU	Operational taxonomic unit
RTF	Reduced transport fluid
SIMS	Secondary ion mass spectrometry
SIP	Isotope-labeled substrates
TGGE	Temperature gradient gel electrophoresis
T-RFLP	Terminal restriction fragment length polymorphism

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## Introduction

We live in a world dominated by microbes [1]. In fact, various environments, including multicellular organisms, are inhabited by a myriad of complex and diversified microbial assemblages. The complete set of microorganisms that resides in a given habitat is referred to as “microbiota” and combines diverse microbial species such as bacteria, viruses, and fungi. Through this chapter, we will be mainly focusing on the bacterial communities that are associated with several human body sites.

## Microbiota Research: From Culture- to Molecular-Based Methods

Until early in the twenty-first century, studies of microbiota were traditionally addressed using culture-dependent methods. Culture of pure microbial colonies using selective and diverse culture media (solid, semisolid, and liquid), which take advantage of the distinctive metabolic properties of the microorganisms, has enabled isolation, identification, and characterization of several microbial species, ultimately defining treatments against many pathogenic microbes [2, 3]. Nowadays, culture methods continue to be an approach in exploring microbial diversity [4–7] and are central for identifying pathogenic organisms from clinical specimens. However, numerous microbial species show fastidious growth