Published in final edited form as:
Curr Infect Dis Rep. 2011 February ; 13(1): 28–34. doi:10.1007/s11908-010-0147-7.

Current Concepts of the Intestinal Microbiota and the Pathogenesis of Infection

Leslie H. Wardwell,
Department of Immunology and Infectious Diseases, Harvard School of Public Health, 665 Huntington Avenue, SPH 1 909, Boston, MA 02115, USA. Harvard Medical School, Boston, MA, USA

Curtis Huttenhower, and
Department of Biostatistics, Harvard School of Public Health, 655 Huntington Avenue, SPH 2, Boston, MA 02115, USA

Wendy S. Garrett
Department of Immunology and Infectious Diseases, Harvard School of Public Health, 665 Huntington Avenue, SPH 1 909, Boston, MA 02115, USA. Department of Genetics and Complex Diseases, Harvard School of Public Health, 665 Huntington Avenue, SPH 1 909, Boston, MA 02115, USA. Harvard Medical School, Boston, MA, USA. Dana Farber Cancer Institute, Boston, MA, USA

Leslie H. Wardwell: wardwell@fas.harvard.edu; Curtis Huttenhower: chuttenh@hsph.harvard.edu; Wendy S. Garrett: wgarrett@hsph.harvard.edu

Abstract

The human gastrointestinal tract is populated by a vast and diverse community of microbes. This gut microbiota participates in host metabolism, protects from invading microbes, and facilitates immune system development and function. In this review, we consider the contributions of intestinal microbes to the pathogenesis of infectious diseases. Key concepts of colonization resistance, host-commensal microbe interaction in immunity, antibiotics and gut bacterial communities, viral-gut bacterial interactions, and evolving methods for studying commensal microbes are explored.

Keywords

Intestine; Commensal; Microbiota; Infection; Gut; Bacteria; Gastrointestinal; Microbial community; Infectious disease

Introduction

The intestinal microbiota, the microbial communities colonizing the small and large bowel, play an important role in a multitude of host functions. Intestinal bacteria function in energy extraction from food, production of key metabolites for host physiology, immune system development, and protection from and response to systemic and gastrointestinal infectious diseases. These communities are in turn shaped by host genetics, diet, and environment and thus exhibit substantial inter-individual variation [1••]. Advances in sequencing technology

Correspondence to: Wendy S. Garrett, wgarrett@hsph.harvard.edu.

Disclosure Conflicts of interest: L. Wardwell—none; C. Huttenhower—none; W. Garrett—none.

and the computational methodology to interpret these data have resulted in an increased appreciation for the role of gut microbiomes in the pathogenesis of obesity [2], inflammatory bowel disease [3], type 1 diabetes [4], and metabolic syndrome [5]. Herein we consider how the intestinal microbiota contributes to susceptibility and response to infectious diseases.

Up to 100 trillion bacteria colonize the human gut, representing an estimated 200 to 1000 distinct bacterial species. (See Table 1 for key terms for the study of the intestinal microbiota.) The intestinal epithelium provides an essential barrier that ensures a separate peace between host and microbe. However, there is constant cross-talk across this barrier between the immune system and microbiota that is equally important for homeostasis in the gut. The epithelium, innate and adaptive immune systems, and the intestinal mucus are all active participants in host defense from infectious diseases that target the gastrointestinal mucosa as their portal to invading their human hosts. Gut bacteria also provide an important line of defense for their hosts against invading pathogens.

Colonization Resistance

Colonization resistance is the process by which the indigenous gut microbiota protects a host from infectious microbes. Several mechanisms underpin colonization resistance, the foremost of which is competition for space and nutrients along the mucus layer and within the lumen. In addition, short-chain fatty acids, which are products of commensal bacterial metabolism, are also bactericidal for some enteric pathogens. Certain *Lactobacilli*, in particular members of the *acidophilus* subgroup, generate reactive oxygen species. Numerous commensals produce bacteriocin and bacteriocin-like molecules as defensive strategies. Bacteriocins fall into three general categories (I, IIa/b/c, and III) and have distinct activities: nucleases, DNAses, cell wall component (eg, peptidoglycan) production inhibitors, and pore formers. Recent studies using mouse models suggest that the composition of a host's microbiota may influence susceptibility to enteropathogens [6•]. Furthermore, particular members of gut microbial communities impact the ability of pathogens to invade and beneficial microbes to colonize. Hosts with high *Escherichia coli* densities are more susceptible to *Salmonella enterica* infection, whereas hosts with high *Lactobacilli* counts are more easily colonized with probiotic strains of *Lactobacilli* [6•]. The relationship between disease susceptibility and features of microbial communities raises the question of how knowledge of microbial communities can be leveraged into improving colonization resistance to prevent and treat infectious diseases.

Commensal Bacteria and the Immune Response

Commensal enteric bacteria are required for immune cell development and function within the gastrointestinal tract and systemically. Studies from gnotobiotic mice (mice lacking endogenous microbes) demonstrate abnormal Peyer's patches, lymph nodes, and spleen; decreased numbers of mucosal and systemic innate and adaptive immune cells; and markedly reduced levels of immunoglobulins and other host defense molecules [7]. Susceptibility to numerous bacterial, viral, and parasitic infections is also increased in germ-free mice, resulting from loss of colonization resistance and impaired immune system responses.

Several recent studies have identified that select species or specific bacterial products play a critical role in the proper functioning of the immune system. Although *Bacteroides fragilis* is well known for its opportunistic role in intraperitoneal abscesses, it also bestows benefits to the host regarding the development of adaptive immunity in the gut and system-wide. A zwitterionic polysaccharide, polysaccharide A, expressed by *B. fragilis* is responsible for these beneficial effects on host immune cell development and homeostasis [8••]. In addition,

polysaccharide A also appears to protect mice from experimentally induced inflammatory bowel disease and central nervous system inflammation [9,10]. T helper 17 cells (Th17), a subset of $CD4^+$ T cells named for its production of the proinflammatory cytokine IL-17, function in host defense against fungi and extracellular bacteria and have been implicated in the pathogenesis of numerous autoimmune diseases [11]. In mice, the induction of intestinal Th17 cells requires that the small intestine is colonized with segmented filamentous bacteria (SFB), a group of anaerobic, spore-forming, gram-positive bacteria [12]. SFB stimulate the expression of several genes involved in antimicrobial defenses and inflammation. The bacterial molecules, host sensors, and signaling pathways involved in this SFB-mediated Th17 development remain to be defined.

Both immune and non-immune system cells express a diverse repertoire of pattern recognition receptors that bind molecular patterns shared among microbes. The toll-like receptors (TLRs) are one family of pattern recognition receptors (select receptors and their ligands are shown in Table 2). DNA from commensal bacteria, which can bind to TLR9, plays an important role in host defenses against microsporidia, a parasite that causes diarrhea in immuno-compromised hosts [13]. Engagement of multiple toll-like receptors by the commensal gut microbiota contributes to the development of protective immune responses against *Toxoplasma gondii* [14]. Flagellin, the TLR5 ligand, recently was shown to restore innate immune defenses against and decrease colonization levels of vancomycin-resistant *Enterococcus* [15]. The nucleotide-binding domain, leucine rich repeat containing proteins, are another family of microbial recognition receptors. Nucleotide-binding oligomerization domain protein (NOD) 2 has a broad specificity for peptidoglycan and NOD1 senses diaminopimelic acid-type peptidoglycan (DAP-PG). Intestinal commensal bacterial-derived DAP-PG plays an important role in priming systemic innate immunity. Specifically, studies of NOD1-deficient and WT mice demonstrated that importance of NOD1 signaling for neutrophil killing of *Streptococcus pneumoniae* and *Staphylococcus aureus* [16•]. Thus, humans and our ancestors evolved a dual-purpose system for sensing microbes—a system that not only senses endogenous microbes for the proper development and functioning of the immune system, but also senses pathogenic invaders to initiate host defense programs.

Antibiotics and the Commensal Microbiota

Infectious disease physicians commonly encounter the disruptive effects of antibiotics on the endogenous flora, the most familiar of which are the antibiotic-associated diarrheas. Recent genomic studies of the intestinal microbiota, both in human populations and mouse models, have revealed that antibiotics have unexpectedly widespread and enduring effects on endogenous gut microbes. Duration of treatment, type of antibiotic, and individual differences all influence antibiotic-induced changes in the composition of the gut bacterial community and the resiliency of gut community members to antibiotic treatment. 16S rDNA enumerations from human stool before and 4 weeks after ciprofloxacin treatment revealed that although the majority of gut microbes eventually returned to their pre-treatment relative abundance levels, taxa belonging to the Clostridiales order did not recover during the observation period [17]. A common theme illuminated by studies of the antibiotic effects on gut microbial composition is a reduction in relative abundance of members of the Firmicutes phylum and specifically several *Lactobacillus* species, many of which are considered beneficial microbes [18,19]. Broad-spectrum antibiotic treatment of mice revealed large proportional increases in the Bacteroidales order. After only 1 day of antibiotics, representation of this order increased to 95%, from basal levels of 60% to 70% of the total bacterial population [18]. These perturbations have the potential to be significant in light of recent studies demonstrating a link between diabetes [20] and obesity [2] and decreased gut microbial diversity and altered Bacteroidetes/Firmicutes ratios.

Antibiotic-induced alterations of the composition or function of gut microbial communities are key effectors in susceptibility to several gastrointestinal bacterial diseases. Antibiotic-associated diarrhea (AAD) is a common complication of antibiotic use, and *Clostridium difficile* is the most frequent cause of AAD, colitis, and pseudomembranous colitis [21]. *Klebsiella oxytoca*, *Clostridium perfringens*, *Salmonella* spp, *Candida* spp, and *Staphylococcus aureus* are far less common causes of AAD. Both the incidence and death rates of *C. difficile*–associated colitis have increased at an alarming rate over the past decade (117% increase in incidence between 2000 and 2006 and a 35% increase in mortality rates) [21]. Estimates of asymptomatic carriage of *C. difficile* range between 1% and 55.4%, based on the population observed [22•]. A few studies have begun to delve into how the composition of the fecal microbiota affects susceptibility and risk of recurrence of *C. difficile*–associated diarrhea (CDAD). A correlation between CDAD and the extent and duration of decreased diversity in response to antibiotics is emerging [22•]. A recent study by Giel et al. [23•] provides mechanistic insight into how antibiotic treatment may result in *C. difficile* sporulation and subsequent AAD. Bile salt metabolism by the commensal microbiota appears to be a key effector. A cholestyramine-sensitive factor in cecal or intestinal extracts of antibiotic-treated mice stimulated *C. difficile* colony formation from spores much more efficiently than samples from untreated mice [23•].

Antibiotics also increase susceptibility to invading microbes of the gastrointestinal tract. Treatment of mice with streptomycin or vancomycin lowered the inoculum dose required to cause morbidity with *Salmonella enterica* Serovar Typhimurium [24]. The doses of antibiotics used in this study did not reduce total bacteria numbers in the gut, a finding which emphasizes that composition and function of the community are important factors in mediating host resistance to invading pathogens. Beyond shifting relative abundances of gut bacteria, antibiotics also have the ability to influence the genomic potential and expression of the gut microbial community. Treatment with high doses of antibiotics selects for bacteria with antibiotic resistance genes on mobile genetic elements. Even though the typical members of the gut microbial community may recolonize the gut over time, horizontal gene transfer of antibiotic resistance may irrevocably alter the intestinal microbiota.

Gut Microbiota as Reservoirs of Resistance

The existence of lag times between when widespread clinical use of an antibiotic begins and when resistance becomes a problem—a few years for penicillin and more than 30 years for vancomycin [25]—suggests that gut bacteria living without exposure to antibiotics do not typically harbor resistance genes. However, bacteria in the soil, an ancient antibiotic resistance reservoir, have spent millions of years evolving in the presence of natural antimicrobials, resulting in the expression and spread of many resistance genes via horizontal gene transfer (HGT) [26•]. The gut represents an environment that, like soil, is highly conducive for HGT—a dense population of bacterial cells, often found organized similar to a biofilm [25]. Once in the gut, antibiotic resistance genes can disseminate throughout the bacterial population, especially under selection pressure from clinically administered antibiotics, and these effects of HGT can endure after selection is removed. After combined antibiotic treatment for *Helicobacter pylori* gastritis (with a clarithromycin and metronidazole regimen), gut enterococci exhibited high macrolide resistance for 1 year after treatment; for one patient, resistance remained even after 3 years [27]. A study of the gut microbiotas of two healthy individuals with no antibiotic exposure in the preceding year found antibiotic resistance genes in both commensal and opportunistic fecal bacteria [28••]. Almost half of the antibiotic resistance genes identified from the sub-cultured fecal isolates were genetically identical to antibiotic resistance genes found in known human pathogens [28••]. Thus, the commensal gut microbiome represents a reservoir of antibiotic-resistance genes. Through HGT, otherwise harmless commensals have the potential to transfer

antibiotic resistance determinants to pathogens, which may lead to the emergence of clinically problematic strains.

Viruses and Gut Bacterial Communities

Viral Gastroenteritis

Viruses are frequently the causative agent of gastroenteritis, one of the most common infectious diseases worldwide [29]. Specifically, noroviruses were found to be responsible for 93% of viral gastroenteritis outbreaks in the United States during a 3-year study period between 1997 and 2000 [30]. Although viral gastroenteritis in adults is most commonly linked to noroviruses, gastroenteritis in children is caused by a broader group of viruses that, along with noroviruses, includes rotaviruses, astroviruses, and sapoviruses [31]. Both astroviruses and rotaviruses have been shown to directly increase gut mucosal permeability [32]. Loss of gut barrier integrity alters the delicate balance between the immune system and commensal microbiota, and thus compromises defenses against a broad range of gastrointestinal pathogens.

The variable attack rates of enteric viruses bring into question the role of the gut microbiota in shaping susceptibility. Undertaking prospective studies of the gut microbiota that follow populations over time may illuminate characteristics of microbial communities associated with resistance to viral gastroenteritis. Although it is not well understood if or how gut microbial populations affect infection with enteric viruses, rehydration therapy in conjunction with supplementation with multiple *Lactobacilli spp (reuteri, acidophilus, delbrueckii bulgaricus,* and *casei*) and *Saccharomyces boulardii* reduced the duration of rotavirus-associated diarrhea and significantly reduced viral shedding [33,34]. Further research into how certain bacteria are able to aid the host in clearance of viral infections could be beneficial not only in understanding viral pathogenesis, but also in understanding relationships between viruses and bacteria in the gut.

Noroviruses and Inflammatory Bowel Disease Susceptibility

Commensal gut bacteria are important contributors to the pathophysiology of inflammatory bowel disease (IBD), and evidence suggests that the gastrointestinal microbiotas of IBD patients differ from healthy controls [3], with reduced relative abundances of members of the *Bacteroidales* and *Lachnospiraceae* families [35]. Recent evidence suggests that the contribution of microbes to IBD extends beyond commensal bacteria to include enteric viruses. About 50% of individuals of European descent carry a mutant allele of the autophagy gene *Atg16L1*, which is a Crohn's disease (CD) susceptibility allele [36]. A recent study using a mouse model with reduced *Atg16L1* function begins to explain the contributions of both viruses and bacteria to CD susceptibility. Infection with a specific norovirus along with a full complement of gut bacteria was required for eliciting CD-like pathology after intestinal injury was triggered in mice with reduced *Atg16L1* [37••]. In the absence of viral exposure or if the commensal bacteria were depleted with antibiotics, CD-like inflammation did not develop, thus demonstrating that a complex web of microbial (viral and bacterial) and host factors underlie CD. Although enteric viruses may not cause IBD single-handedly, they have the potential to shape the host immune response and intestinal environment in a manner that promotes dysfunctional host-commensal interactions.

Gut Bacterial Viruses

Studies of human gut microbiota have revealed that coliphages (enteric bacterial viruses) infect many *Enterobacteriaceae* (*E. coli*, *Salmonella* spp, *Klebsiella* spp) and other Bacteroidetes and Firmicutes [38]. Despite overwhelming evidence of viral-bacterial

interactions in the human gut, the consequences of such interactions in the context of gastrointestinal disease have not been fully explored. Classical phage-bacterial host interactions are characterized by an evolutionary struggle for and against viral invasion. However, in the fecal microbial community of healthy humans, viral life cycles tend to be temperate (non-lytic) with low variation over time, and with little genomic evidence of bacteria evolving mechanisms to prevent phage attachment and invasion [39••]. Enteric phages that are in constant contact with commensal bacteria in the gut have the potential to act in a commensal manner of their own and may confer growth advantages to their hosts [38]. Several viral-like particles found in healthy human feces encode proteins involved in peptidoglycan synthesis, pyruvate and folate metabolism, and oxidative stress response [39••]. By introducing new genes and associated functions, viruses have the ability to change the microbiome of the gut. In light of recent findings that recognize associations between bacteria and human diseases, and between bacteria and viruses in the gut, future attention should be focused on the ways in which coliphages are able to shape the composition and function of commensal bacterial communities in the gastrointestinal tract.

Gut Microbes and HIV

HIV is a viral infection that illustrates the importance of gut microbes for the pathogenesis of infectious disease. Mucosal memory $CD4^+$ T cells, the bulk of which reside in the gastrointestinal tract, are an early target of HIV during acute infection [40]. Although the exact mechanisms remain unclear, HIV-infected individuals can exhibit numerous gastrointestinal tract pathologies. These immunological and epithelial barrier abnormalities contribute to the enteropathies, malabsorption syndromes, and gastrointestinal infections observed in this population [41]. In chronic phases of human HIV infection and nonhuman primate SIV infection, impaired gut epithelial barrier integrity results in high levels of serum lipopolysaccharide (LPS) due to translocation of commensal bacteria across the intestinal mucosa [42]. In mouse models, LPS serum levels observed in HIV infection are comparable to levels observed during acute colitis [43]. Gut microbes and aberrant responses to them by the HIV-altered mucosal system may play a role in the systemic inflammation/immune activation implicated in HIV disease progression [44]. Studies of the respiratory and distal gut microbiomes of HIV-infected individuals are an active area of investigation that hopefully will help identify both commensal microbe-based predictive biomarkers of disease progression and strategies to promote health.

Evolving Methods for Studying the Commensal Microbiota

The majority of our intestinal microbes are not easily identified using standard culturing techniques, and high-throughput sequencing technologies generate vast datasets describing these diverse microbial communities. Bioinformatics are thus an essential part of any study that investigates the intestinal microbiota [45••]. Because high-throughput sequencing is typically used to quantify taxonomic markers such as the 16S ribosomal subunit gene [46] or metagenomic fragments [47], standard data processing steps have been developed to filter technical artifacts and remove chimeric sequences [48]. As with any modern sequencing project, the size of metagenomic data for more than a few samples can be immense, and plans should be made up front for bioinformatic processing and analysis time. Several computational pipelines are emerging for subsequently viewing this sequence data in terms of community diversity [48], the specific organisms present in a sample [49], or functional and metabolic activity [50]. Once determined, any of these features can be used to better understand the biomolecular mechanisms linking a community with its host environment, or they can be used as high-dimensional biomarkers in a manner similar to gene expression microarrays or genome-wide association studies.

Conclusions

We live in symbiosis with an immense microbial world that inhabits the gastrointestinal tract and other surfaces of the human body. Although there has been a long-standing appreciation for the benefits microbes may confer on their human hosts, only recently have details of these complex relationships begun to emerge. Several families of receptors that mediate host-microbial interactions have been characterized, and undoubtedly more will be discovered. Specific bacterial species and the bacterial molecules that direct the development of immunity and regulate immune cell homeostasis are coming to light. Sequencing technologies and the bioinformatic techniques to interpret these data are facilitating a conceptual shift in thinking of microbes not as single species, but as complex communities with interdigitating and diverse functions. The collective metagenome of the gut microbiota is prodigious and constitutes an environmental force that influences the function of cells and tissues within and beyond the gastrointestinal tract. Inter-individual differences in the clinical spectrum of response to acute and chronic infectious diseases are beginning to be linked to the gastrointestinal microbiota. However, a nuanced understanding of the association between the intestinal microbiota and the pathogenesis of infectious diseases is far from complete. The tools for such studies are evolving at a rapid rate, and knowledge of microbial communities is likely to be actualized into preventing and treating infectious diseases in the near future.

References

Papers of particular interest, published recently, have been highlighted as:

• Of importance

•• Of major importance

1••. Frank DN, Pace NR. Gastrointestinal microbiology enters the metagenomics era. Curr Opin Gastroenterol. 2008; 24(1):4–10. An excellent review summarizing metagenomic techniques and their application to studies of human health and disease. [PubMed: 18043225]

2. Turnbaugh PJ, Hamady M, Yatsunenko T, et al. A core gut microbiome in obese and lean twins. Nature. 2009; 457(7228):480–4. [PubMed: 19043404]

3. Qin J, Li R, Raes J, et al. A human gut microbial gene catalogue established by metagenomic sequencing. Nature. 2010; 464:59–65. [PubMed: 20203603]

4. Wen L, Ley RE, Volchkov PY, et al. Innate immunity and intestinal microbiota in the development of Type 1 diabetes. Nature. 2008; 455(7216):1109–13. [PubMed: 18806780]

5. Vijay-Kumar M, Aitken JD, Carvalho FA, et al. Metabolic syndrome and altered gut microbiota in mice lacking Toll-like receptor 5. Science. 2010; 328(5975):228–31. [PubMed: 20203013]

6•. Stecher B, Chaffron S, Käppeli R, et al. Like will to like: abundances of closely related species can predict susceptibility to intestinal colonization by pathogenic and commensal bacteria. PLoS Pathogens. 2010; 6:e1000711. An investigation of the mechanism underpinning colonization resistance using a mouse model of infection with Salmonella enterica. [PubMed: 20062525]

7. Macpherson AJ, Harris NL. Interactions between commensal intestinal bacteria and the immune system. Nature reviews Immunology. 2004; 4:478–85.

8••. Round JL, Mazmanian SK. The gut microbiota shapes intestinal immune responses during health and disease. Nature Reviews Immunology. 2009; 9:313–23. A thorough review of the role of gut commensal bacteria in immune system development and response.

9. Mazmanian SK, Round JL, Kasper DL. A microbial symbiosis factor prevents intestinal inflammatory disease. Nature. 2008; 453 (7195):620–5. [PubMed: 18509436]

10. Ochoa-Reparaz J, Mielcarz DW, Wang Y, et al. A polysaccharide from the human commensal Bacteroides fragilis protects against CNS demyelinating disease. Mucosal Immunol. 2010; 3(5): 487–95. [PubMed: 20531465]

11. Korn T, Bettelli E, Oukka M, Kuchroo VK. IL-17 and Th17 Cells. Annual review of immunology. 2009; 27:485–517.
12. Ivanov II, Atarashi K, Manel N, et al. Induction of intestinal Th17 cells by segmented filamentous bacteria. Cell. 2009; 139:485–98. [PubMed: 19836068]
13. Hall JA, Bouladoux N, Sun CM, et al. Commensal DNA limits regulatory T cell conversion and is a natural adjuvant of intestinal immune responses. Immunity. 2008; 29(4):637–49. [PubMed: 18835196]
14. Benson A, Pifer R, Behrendt CL, et al. Gut commensal bacteria direct a protective immune response against Toxoplasma gondii. Cell host & microbe. 2009; 6:187–96. [PubMed: 19683684]
15. Kinnebrew MA, Ubeda C, Zenewicz LA, et al. Bacterial flagellin stimulates Toll-like receptor 5-dependent defense against vancomycin-resistant Enterococcus infection. J Infect Dis. Feb 15; 201(4):534–43. [PubMed: 20064069]
16•. Clarke TB, Davis KM, Lysenko ES, et al. Recognition of peptidoglycan from the microbiota by Nod1 enhances systemic innate immunity. Nature Medicine. 2010; 16:228–31. A recent paper demonstrating the role of commensal bacterial products for robust system immune function.
17. Dethlefsen L, Huse S, Sogin ML, Relman DA. The pervasive effects of an antibiotic on the human gut microbiota, as revealed by deep 16S rRNA sequencing. PLoS Biol. 2008; 6(11):e280. [PubMed: 19018661]
18. Sekirov I, Tam NM, Jogova M, et al. Antibiotic-induced perturbations of the intestinal microbiota alter host susceptibility to enteric infection. Infection and immunity. 2008; 76:4726–36. [PubMed: 18678663]
19. Hill DA, Hoffmann C, Abt MC, et al. Metagenomic analyses reveal antibiotic-induced temporal and spatial changes in intestinal microbiota with associated alterations in immune cell homeostasis. Mucosal immunology. 2010; 3:148–58. [PubMed: 19940845]
20. Giongo A, Gano KA, Crabb DB, et al. Toward defining the autoimmune microbiome for type 1 diabetes. ISME J. Jul 8.
21. Onderdonk, AB.; Garrett, WS. Gas Gangrene and Other Clostridium-Associated Diseases. In: Mandell, GL.; Bennett, JE.; Dolin, R., editors. Mandell, Douglas, and Bennett's Principles adn Practice of Infectious Disease. 7. Vol. 2. Philadelphia: Churchill, Livingstone, Elsevier; 2010. p. 3103-9.
22•. Walk ST, Young VB. Emerging insights into antibiotic-associated diarrhea and Clostridium difficile infection through the lens of microbial ecology. Interdisciplinary perspectives on infectious diseases. 2008; 2008:125081. An interesting review of Antibiotic-associated diarrhea and Clostridium difficile. [PubMed: 19277109]
23•. Giel JL, Sorg JA, Sonenshein AL, Zhu J. Metabolism of bile salts in mice influences spore germination in Clostridium difficile. PloS one. 2010; 5:e8740. A recent paper providing insight into the contribution of commensal microbes to Clostridium difficile sporulation. [PubMed: 20090901]
24. Sekirov I, Finlay BB. The role of the intestinal microbiota in enteric infection. The Journal of physiology. 2009; 587:4159–67. [PubMed: 19491248]
25. Marshall BM, Ochieng DJ, Levy SB. Commensals: Underappreciated Reservoir of Antibiotic Resistance. Microbe. 2009; 4:231–8.
26•. Allen HK, Donato J, Wang HH, et al. Call of the wild: antibiotic resistance genes in natural environments. Nature Reviews Microbiology. 2010; 8:251–9. A comprehensive review of the types and functions of antibiotic resistance genes found in environmental microbes.
27. Sjolund MS, Andersson DI, Blaser MJ, Engstrand A. Brief Communication Long-Term Persistence of Resistant Enterococcus Species after Antibiotics To Eradicate Helicobacter pylori. Annals of Internal Medicine. 2003:483–8. [PubMed: 13679325]
28••. Sommer MO, Dantas G, Church GM. Functional characterization of the antibiotic resistance reservoir in the human microflora. Science (New York, NY). 2009; 325:1128–31. This article demonstrates that the gut microbiome represents a key reservoir for antibiotic resistance genes.
29. Bobak, DA.; Guerrant, RL. Nausea, Vomiting, and Noninflammatory Diarrhea. In: Mandell, GL.; Bennett, JE.; Dolin, R., editors. Mandell, Douglas, and Bennett's Principles and Practice of Infectious Disease. 7. Vol. 1. Philadelphia: Churchill, Livingstone, Elsevier; 2010. p. 1359-73.

30. Fankhauser RL, Monroe SS, Noel JS, et al. Epidemiologic and molecular trends of “Norwalk-like viruses” associated with outbreaks of gastroenteritis in the United States. The Journal of infectious diseases. 2002; 186:1–7. [PubMed: 12089655]

31. Lyman WH, Walsh JF, Kotch JB, et al. Prospective study of etiologic agents of acute gastroenteritis outbreaks in child care centers. The Journal of pediatrics. 2009; 154:253–7. [PubMed: 18783794]

32. Moser LA, Carter M, Schultz-Cherry S. Astrovirus increases epithelial barrier permeability independently of viral replication. Journal of virology. 2007; 81:11937–45. [PubMed: 17699569]

33. Fang S-B, Lee H-C, Hu J-J, et al. Dose-dependent effect of Lactobacillus rhamnosus on quantitative reduction of faecal rotavirus shedding in children. Journal of tropical pediatrics. 2009; 55:297–301. [PubMed: 19203988]

34. Vandenplas Y, Salvatore S, Vieira M, et al. Probiotics in infectious diarrhoea in children: are they indicated? European journal of pediatrics. 2007; 166:1211–8. [PubMed: 17611775]

35. Frank DN, St Amand AL, Feldman RA, et al. Molecular-phylogenetic characterization of microbial community imbalances in human inflammatory bowel diseases. Proceedings of the National Academy of Sciences of the United States of America. 2007; 104:13780–5. [PubMed: 17699621]

36. Barrett JC, Hansoul S, Nicolae DL, et al. Genome-wide association defines more than 30 distinct susceptibility loci for Crohn’s disease. Nat Genet. 2008; 40(8):955–62. [PubMed: 18587394]

37••. Cadwell K, Patel KK, Maloney NS, et al. Virus-plus-susceptibility gene interaction determines Crohn’s disease gene Atg16L1 phenotypes in intestine. Cell. 2010; 141(7):1135–45. This article provides insight into the contribution of genes and environment to the pathophysiology of Crohn’s disease by revealing links between the host Atg16L1 gene, specific norovirus infection, and bacterial gut communities. [PubMed: 20602997]

38. Letarov A, Kulikov E. The bacteriophages in human- and animal body-associated microbial communities. Journal of applied microbiology. 2009; 107:1–13. [PubMed: 19239553]

39••. Reyes A, Haynes M, Hanson N, et al. Viruses in the faecal microbiota of monozygotic twins and their mothers. Nature. 2010; 466(7304):334–8. This study of monozygotic twins and their mothers describes the diversity and variation of fecal phage viromes in healthy individuals. [PubMed: 20631792]

40. Brenchley JM, Schacker TW, Ruff LE, et al. CD4+ T cell depletion during all stages of HIV disease occurs predominantly in the gastrointestinal tract. The Journal of experimental medicine. 2004; 200:749–59. [PubMed: 15365096]

41. Douek D. HIV disease progression: immune activation, microbes, and a leaky gut. Topics in HIV medicine: a publication of the International AIDS Society, USA. 2007; 15:114–7.

42. Brenchley JM, Price DA, Schacker TW, et al. Microbial translocation is a cause of systemic immune activation in chronic HIV infection. Nature medicine. 2006; 12:1365–71.

43. Hofer U, Schlaepfer E, Baenziger S, et al. Inadequate clearance of translocated bacterial products in HIV-infected humanized mice. PLoS pathogens. 2010; 6:e1000867. [PubMed: 20442871]

44. Nazli A, Chan O, Dobson-Belaire WN, et al. Exposure to HIV-1 directly impairs mucosal epithelial barrier integrity allowing microbial translocation. PLoS pathogens. 2010; 6:e1000852. [PubMed: 20386714]

45••. Hamady M, Knight R. Microbial community profiling for human microbiome projects: tools, techniques, and challenges. Genome Res. 2009; 19(7):1141–52. A thorough review of sequencing techniques, analytic methods, and experimental design for studies directed at the human microbiome. [PubMed: 19383763]

46. Tringe SG, Hugenholtz P. A renaissance for the pioneering 16S rRNA gene. Curr Opin Microbiol. 2008; 11(5):442–6. [PubMed: 18817891]

47. Peterson DA, Frank DN, Pace NR, Gordon JI. Metagenomic approaches for defining the pathogenesis of inflammatory bowel diseases. Cell Host Microbe. 2008; 3(6):417–27. [PubMed: 18541218]

48. Caporaso JG, Kuczynski J, Stombaugh J, et al. QIIME allows analysis of high-throughput community sequencing data. Nat Methods. 2010; 7(5):335–6. [PubMed: 20383131]

49. Meyer F, Paarmann D, D'Souza M, et al. The metagenomics RAST server—a public resource for the automatic phylogenetic and functional analysis of metagenomes. BMC Bioinformatics. 2008; 9:386. [PubMed: 18803844]

50. Mitra S, Klar B, Huson DH. Visual and statistical comparison of metagenomes. Bioinformatics. 2009; 25(15):1849–55. [PubMed: 19515961]

Table 1

Key terms for study of the intestinal microbiota

16S rRNA gene	A gene expressed by both Bacteria and Archaea that encodes a subunit of the 30S ribosome. Sequencing of 16S rRNA gene is frequently used in phylogenetic studies of the microbiota. Hypervariable regions of this gene facilitate taxonomic assignment.
Operational taxonomic unit (OTU)	A term used in taxonomic assignments. A group of like microbial sequences. OTUs with ≥97% identity are considered to be of the species.
Microbiome	A specific community of microbes and their environment (eg, a particular body site).
Microbiota	The microbes living within a given environment (eg, the gut).
Metagenome	The total collection of genomes from a community (eg, from microbes from human fecal samples).
Bacteroidetes	One of the two most abundant bacterial phyla in the human intestinal contents
Firmicutes	One of the two most abundant bacterial phyla in the human intestinal contents

Table 2

Select pattern recognition receptors and their microbial ligands

Receptor	Ligand
Toll-like receptor 1	Triacyl lipoproteins
Toll-like receptor 2	Glycolipids, lipoproteins, lipoteichoic acid, hsp70, β-glucan
Toll-like receptor 3	Double-stranded RNA
	Poly I:C
Toll-like receptor 4	Lipopolysaccharide
Toll-like receptor 5	Flagellin
Toll-like receptor 6	Diacyl lipoproteins
Toll-like receptor 7	Single-stranded RNA
Toll-like receptor 8	Single-stranded RNA
Toll-like receptor 9	Unmethylated CpG oligonucleotides
Toll-like receptor 10	Unknown
Toll-like receptor 11	Profilin
Toll-like receptor 12, 13, 15	Unknown
NOD1	Diaminopimelic acid-type peptidoglycan
NOD2	Peptidoglycan

NOD nucleotide-binding oligomerization domain protein