Published in final edited form as:

J Gastrointest Surg. 2018 June ; 22(6): 1112–1123. doi:10.1007/s11605-018-3755-4.

Current State of Knowledge on Implications of Gut Microbiome for Surgical Conditions

Edmund B. Chen, MD¹, Cori Cason, MD¹, Jack A. Gilbert, PhD², and Karen J. Ho, MD¹

¹Department of Surgery, Northwestern University Feinberg School of Medicine

²Department of Surgery, University of Chicago

Abstract

The role of the microbiome in human health has become a central tenant of current medical research, infiltrating a diverse disciplinary base whereby microbiology, computer science, ecology, gastroenterology, immunology, neurophysiology and psychology, metabolism, and cardiovascular medicine all intersect. Traditionally, commensal gut microbiota have been assumed to play a significant role only in the metabolic processing of dietary nutrients and host metabolites, the fortification of gut epithelial barrier function, and the development of mucosal immunity. However, over the last 20 years, new technologies and renewed interest has uncovered a considerably broader influence of the microbiota on health maintenance and disease development, many of which are of particular relevance for surgeons. This article provides a broad overview of the current state of knowledge and a review of the technology that helped in their formation.

Keywords

microbiota; gastrointestinal tract; medicine

INTRODUCTION

Human microbes, consisting of bacteria, fungi, archaea, and viruses, are at least as abundant as the somatic cells of the body.¹ Microbes colonize every surface of the body that is exposed to the external environment, including the skin, genitourinary, gastrointestinal, and respiratory tracts (reviewed in Cho and Blaser 2012²). The gut microbiome, a term for the collective community of microbial species and their genomic content, dwarfs the human genome by 150-fold, with an estimated 3.3 million microbial genes.^{3–5} Over the past 25 years, advances in culture-independent techniques, next-generation sequencing, and bioinformatics have paved the way for an explosion of new knowledge regarding the gut microbial ecosystem and its effect on the host. This review strives to summarize for the general surgical community the terminology and tools (TABLE 1)^{6–9} used in microbiome

Corresponding author (to whom reprint requests should be sent): Karen J. Ho, MD Division of Vascular Surgery, Northwestern University Feinberg School of Medicine 676 North St. Clair Street, Suite 650 Chicago, IL 60611 Phone number: 312-695-4952 Fax number: 312-695-4955 kho1@nm.org.

Author contributions: All authors contributed to the conception, acquisition, and analysis of data; drafting and critical revision of the manuscript; gave final approval to the manuscript; and accept accountability for all aspects of the work.

medicine and the impact of the gut microbiome on surgical **conditions**, including obesity, non-alcoholic fatty liver disease (NAFLD), colorectal cancer (CRC), intestinal anastomotic leaks, inflammatory bowel disease (IBD), and atherosclerosis (TABLE 2).^{10–22} Due to space limitations, references to in-depth review articles pertaining to each topic are also provided.

SURGICAL CONDITIONS AFFECTED BY THE GUT MICROBIOME

Obesity

The first studies suggesting a relationship between the gut microbiome and obesity were conducted in mice. In 2005, Ley *et al.* compared the microbiota of lean mice to genetically obese leptin knockout (*ob/ob*) mice.²³ *Ob/ob* mice had a 50% reduction in intestinal Bacteroidetes species relative to their lean mouse counterparts, with a concordant 20% increase in the percentage of bacterial taxa associated with the phylum Firmicutes. The shifts in microbial community structure also reflected a change in microbial metabolic potential so that the microbiome of *ob/ob* mice had a greater diversity of encoding enzymes that break down indigestible polysaccharides, *e.g.* fiber, and as a result, *ob/ob* mice have significantly fewer calories remaining in their feces when compared to lean mice.²⁴ When germ-free mice (which are born and raised in sterile conditions and have no commensal organisms) underwent fecal microbiota transplantation from *ob/ob* or lean mouse donors, mice colonized with an *ob/ob* microbiome demonstrated a greater increase in body fat over two weeks than mice colonized with a lean microbiome, despite similar chow consumption between the two groups. Thus, not only was the gut microbiome in the obese mice more efficient in caloric extraction, but an obesity phenotype could be conferred to a mouse host solely through the transplantation of these more calorically efficient gut microbes.²⁴

Bacteroidetes and Firmicutes phyla are also dominant in the human gut microbiome. As in obese mice, obese human subject stool, analyzed with 16S ribosomal RNA (16S rRNA) amplicon sequencing, were found to have relatively fewer Bacteroidetes and more Firmicutes.²⁵ In addition, the diversity of gut microbiota, not just the composition, has been linked to obesity. In a comparison of obese and non-obese Danish individuals, those with reduced microbial species diversity (*i.e.*, microbial gene counts below 480,000; median 600,000) had more marked adiposity, insulin resistance, leptin resistance, and dyslipidemia compared to their counterparts with high gene counts.²⁶ Obese individuals with low gene counts also tended to gain more weight over time compared to those with high gene counts, suggesting that low gut bacterial richness identifies a subset of patients at greater risk for obesity and obesity-related co-morbidities.

Gut microbes also affect obesity through the metabolism of nutrients. Hippurate, which is derived from microbial metabolism of dietary polyphenols, may be linked to *Eubacterium dolichum* and visceral fat mass.²⁷ In addition, gut microbial influence on the circadian clock, which regulates diurnal oscillations in biological processes such as feeding and the concentrations of microbe-derived metabolites, may contribute to diet-induced obesity.²⁸

It follows that if there is a specific subset of gut microbiota that is linked to obesity, there should be a microbial profile associated with leanness. Bacterial strains that have been associated with leanness are *Akkermansia muciniphila*, *Facealibacterium prausnitzii*, and

Bacteroides thetaiotaomicron.^{29,30} Emerging metabolomics data has also shown that polyphenols from cranberry and pomegranate, resveratrol, and gluco-oligosaccharide are protective of obesity on high fat diets and act potentially through beneficial changes to the gut microbiome.^{31–33}

The gut microbiome also appears to be involved in the mechanism behind leanness and weight loss after bariatric surgery. Bariatric surgery is a valid means of achieving major and sustained weight reduction. Common bariatric procedures have traditionally been defined as purely restrictive (*e.g.*, adjustable gastric band or sleeve gastrectomy) or both restrictive and malabsorptive (*e.g.*, Roux-en-y gastric bypass [RYGB] or biliopancreatic diversion with duodenal switch).³⁴ However, the mechanism by which bariatric surgery mitigates obesity and its associated comorbidities involves more than the physical restriction of food intake or reduction of nutrient absorption capacity. Rather, broad changes in host metabolism and energy homeostasis occur after these surgeries, promoting sustained weight loss and resolution of the metabolic syndrome. While the mechanism by which the improved metabolic profile occurs is not completely understood, it has been hypothesized that changes in the gut microbiome resulting from alterations in gut luminal content seen after bariatric surgery modulate some of these host metabolic changes.

Animal models provide strong evidence that changes in the gut microbiome potentiate the effects of bariatric surgery. When obese mice underwent either RYGB surgery or sham surgery, RYGB mice had increased and sustained weight loss, with preferential loss of fatty mass and maintenance of lean mass.³⁵ Post-RYGB mice also had distinct microbiome profiles compared to their sham-operated counterparts. The transfer of the microbiome from RYGB mice into germ-free mice was sufficient to induce weight loss in the recipients **and resulted in increased adiposity in the recipients, however without significant overall weight change**.³⁵

A similar microbiome transplantation study was carried out using the human gut microbiome.³⁶ 16S rRNA amplicon sequencing analysis of fecal samples from a cohort of females who had been randomly assigned to undergo RYGB or vertical banded gastroplasty nine years previously demonstrated that both procedures resulted in similar microbial community profiles that were distinct from profiles in non-operated obese women.³⁶ When stool from post-bariatric surgery patients and non-operated obese patients were transferred to germ-free mice, mice colonized with post-RYGB or post-gastroplasty microbes had decreased fat mass (despite similar body weight gain and food intake) compared to mice that received the microbes from obese, non-operated patients.³⁶ Colonization with post-RYGB microbes notably resulted in the largest increase in lean body mass in the recipient mice.³⁶ These experiments thus demonstrated that the human gut microbiome could directly potentiate the reduction of adiposity seen after bariatric surgery.

Mechanistically, bariatric surgery seems to restore the gut microbiome of obese individuals to that found in their lean counterparts. In a longitudinal human study of patients undergoing RYGB, microbes in the Bacteroidetes phylum were again found to be reduced in obese individuals prior to surgery.³⁷ However, three months after RYGB, the relative abundance of this phylum was restored back to levels closely matching that of lean control subjects. An

increase in Bacteroidetes after surgery correlated with increased reduction in body fat mass and increased serum leptin concentration. In addition, a comparison of 16S rRNA sequences from the stool of normal weight, morbidly obese, and post-RYGB humans demonstrated that there were distinct differences in the bacterial divisions between the three cohorts.³⁸ Notably, methanogenic, or methane-producing, archaea (one of the 3 main kingdoms of life—bacteria, archaea and eukarya) were highly prevalent in obese individuals, but were below the level of detection in normal weight or all-but-one post-RYGB patient. Methanogens control the fermentation of dietary fiber by consuming hydrogen and acetate during methanogenesis, which supports the fermentative process by removing these secondary byproducts, making the fermentation of fiber more energetically favorable for those bacteria capable of the process. As mentioned above, the increase in fiber fermentation allows for the additional extraction of calories from these otherwise indigestible polysaccharides.³⁹ As proof of concept, colonization of mice with methanogens, specifically *Methanobrevibacter smithii*, induced adiposity in the mouse host.³⁹

These changes in the gut microbiome are also seen after other types of bariatric surgery. Sleeve gastrectomies also drive persistent and significant changes in the gut microbiome, as shown in diet-induced obese mice that underwent sleeve gastrectomy and had a significant and sustained expansion of Bacteroidetes and a decrease in Firmicutes.⁴⁰ This expansion of species diversity within the phylum Bacteroidetes matched the post-RYGB results detailed above and correlated with weight loss and improved insulin resistance.⁴⁰ In addition, microbial metabolic functional potential related to host metabolism, such as carbohydrate fermentation, citrate cycle, and the production of amino acids, as determined by shotgun metagenomic sequencing, became more similar to lean controls in obese individuals three months after sleeve gastrectomy.

Taken together, these studies demonstrate that obesity is linked to a unique gut microbiome profile that confers the host with an increased capacity for caloric extraction. Bariatric surgery, in turn, seems to restore a healthier microbiome with a leaner metabolic profile, and this re-alignment of the microbiome potentially contributes to the reduced adiposity, increase in lean mass, and resolution of co-morbidities seen in post-surgery patients. However, the mechanisms by which microbes and microbial by-products affect obesity remain poorly understood and microbiome manipulations that exploit the host-bacteria interaction for the treatment or prevention of obesity still need to be developed. For further information on this topic, readers are directed to a comprehensive review paper¹⁰ on links between the gut microbiome and obesity and the changes induced by bariatric surgery.

Non-Alcoholic Fatty Liver Disease (NAFLD)

NAFLD affects approximately one-third of American adults⁴¹ and is defined by steatosis in the liver of individuals who drink little to no alcohol. Of those with NAFLD, approximately 25% go on to develop its more progressive subtype, non-alcoholic steatohepatitis (NASH). NASH is marked by a chronic inflammatory response to fat accumulation within the liver that progresses to chronic intrahepatic scarring and fibrosis, which in turn, can eventually lead to cirrhosis, hepatocellular carcinoma, and/or the need for liver transplantation.

There are multiple proposed mechanisms for the role of the gut microbiome in the development of NAFLD. One such mechanism involves the interaction between the liver and microbial metabolism of enteric substrates such as ethanol, choline, short chain fatty acids (SCFA), and bile acids.¹² Below, we will discuss ethanol and choline, as a comprehensive review of SCFA and bile acids in NAFLD has been reviewed elsewhere.^{13, 14}

Ethanol and its metabolites such as acetaldehyde, induce triglyceride accumulation in hepatocytes and generate damaging reactive oxygen species,¹⁴ leading to alcoholic liver disease. However, not all ethanol is derived from dietary sources. The microbiome is a significant producer of non-dietary ethanol; one gram of *Escherichia coli* (*E. coli*) is capable of producing up to 0.8 g of ethanol per hour in anaerobic conditions.⁴² Correspondingly, dysbiosis can favor ethanol-producing species. NASH patients have an over-representation of ethanol-producing bacteria such as *E. coli* and correspondingly higher baseline blood alcohol levels than their obese-non NASH or lean counterparts.⁴³ Genetically obese leptin knockout mice also have higher levels of ethanol detected via breath test than their lean counterparts, a difference that is ameliorated with antibiotic treatment.⁴⁴ In rodents, reducing the endogenous microbial production of ethanol with antibiotic decontamination of the gut has been shown to protect against the development of NAFLD.⁴⁵

Choline is another metabolite that has a role in the development of NAFLD and NASH. Choline is produced both endogenously in the liver and obtained from the diet; major dietary sources include fish, eggs, red meats, milk, and poultry.⁴⁶ Choline is required by the liver to generate and secrete very-low-density lipoprotein (VLDL), which in turn, is involved in transporting triglycerides out of the liver into the bloodstream and elsewhere in the body. Deficiency in choline, and therefore VLDL, leads to increased deposition of triglycerides in hepatocytes and steatosis. As such, a model of NASH cirrhosis can be induced in rats with a choline-deficient diet.⁴⁷ Changes in the functional potential of the gut microbiome can lead to choline-deficiency and therefore predispose to NAFLD and NASH. A high fat diet increases microbial hydrolysis of choline into methylamine, which reduces circulating choline concentration to levels found in mice on low-choline diets.⁴⁸ Hence, microbial metabolism of choline is thought to decrease the bioavailability of choline for the human host and disrupt the production of VLDL.

Another proposed mechanism for the microbiome's role in the pathogenesis of NAFLD is related to dysregulation of host intestinal permeability. The gut epithelium serves as a barrier between the non-sterile luminal contents with its diverse microbiome community and the sterile host environment. Components of this barrier include the gut epithelium, *i.e.*, tight junctions between adjacent intestinal epithelial cells, and the gut immune system that is tolerant to the commensal gut organisms.⁴⁹ This barrier can be modulated by changes in the gut microbiome. In mice, disruption of the intestinal barrier potentiates NASH cirrhosis.⁵⁰ Mechanistically, increased intestinal permeability exposes the portal circulation to pro-inflammatory products such as lipopolysaccharide (LPS) derived from gram-negative microorganisms.¹³ Toll-like receptors on hepatic Kupffer cells and stellate cells recognize and are activated by these microbial products, initiating a cascade of cytokine release that leads to inflammation, fibrosis, and eventually cirrhosis.⁵¹

A study in humans compared gut permeability (evaluated by urinary clearance of the orally ingested radioisotope, ^{51}Cr -EDTA) and histology of duodenal tight junctions in biopsy-proven NAFLD patients (n=35) with healthy controls (n=24).⁵² Patients with NAFLD were found to have significantly increased permeability versus healthy controls due to disruption of the intestinal tight junctions and intestinal bacterial overgrowth as measured by glucose breath testing.⁵² The reader interested in learning more about the complex interaction between the gut microbiome and liver disease is directed towards a comprehensive review by Leung *et al.*¹³

Colorectal Cancer (CRC)

Worldwide, CRC represents the second and third most commonly diagnosed cancer in females and males, respectively. Coincidentally, many of the lifestyle factors influencing CRC—age, diet, prominent red meat and fat consumption, obesity, and alcohol and tobacco use—also shape the composition of gut microbiota. Microbial communities and their metabolite products modulate the host immune response, altering epithelial cell signaling and growth, which can promote oncogenesis.¹⁵

Research on the interplay between the gut microbiome and CRC has focused on both specific bacterial strains and broad taxonomic associations. Precedents for a single microorganism playing a role in cancer development include causative roles for *Helicobacter pylori* in the development of gastric cancer, hepatitis B and C viruses in hepatocellular carcinoma, and human papillomavirus in cervical cancer. While a detailed summary of all individual bacterial strains that have been linked to CRC is beyond the scope of this article, (the reader is directed to a comprehensive review by Sears *et al.*¹⁵), it is interesting to highlight some of these proposed species and mechanisms.

Streptococcus bovis endocarditis has classically been associated with colon cancer. Presumably, a breach in colonic mucosal integrity secondary to malignancy allows *S. bovis* to escape the confines of the colon and travel systemically to the heart.⁵³ However, there are contemporary data demonstrating that *S. bovis*, now termed *Streptococcus gallolyticus*, is not just an opportunistic pathogen, but has a direct role in oncogenesis.⁵⁴ Significantly, *S. gallolyticus* member bacteria (SGMB) colonization has been detected in both tumor and non-tumor tissues of CRC patients, with or without a history of bacteremia, especially when compared to non-CRC control patients.⁵⁵ SGMB colonization was highly associated with IL-1 and COX-2 gene expression in colorectal tumors, suggesting that SGMB may have a chronic inflammatory effect in the colon which could promote malignant or premalignant lesions.⁵⁵ Another well-known commensal organism, *Enterococcus faecalis*, produces superoxide radicals, triggering colon macrophages to produce clastogens (or chromosomal-breaking factors) that diffuse through the local area, leading to DNA damage and chromosomal instability in neighboring colonic cells (the “bystander effect”).⁵⁶ Enterotoxigenic *Bacteroides fragilis*-induced carcinogenesis has been linked to its virulence factor, the *B. fragilis* toxin (BFT). BFT, a metalloprotease, cleaves E-cadherin, a tumor suppressor protein, which subsequently leads to enhanced tumor growth through enhanced β -catenin/Wnt signaling.⁵⁷ Select strains of *E. coli* produce colibactin, a small molecule that

crosslinks DNA and leads to DNA damage and could contribute to inflammation-induced CRC.⁵⁸

Metabolites derived from microbial metabolism of dietary nutrients and substrates in the colon also effect colonic health and oncogenic potential. For example, SCFA, which are produced by gut microbes through the fermentation of fiber, have been found to be anti-carcinogenic by promoting an anti-inflammatory state and increasing the apoptosis of colorectal cancer cells (see comprehensive review in ref¹⁶). The breakdown of bile acids by gut microbes has also been linked to colorectal cancer formation. Diets high in saturated fats increase bile acid production,⁵⁹ and population studies have linked diets high in saturated fats to the development of colorectal cancer.⁶⁰ Microbes are responsible for the deconjugation of bile acids to produce secondary bile acids. As detailed in the review by Sears *et al.*,¹⁵ these secondary bile acids (*i.e.*, lithocholic and deoxycholic acid) are potentially carcinogenic in rodent and human studies by inducing a pro-inflammatory state through the production of reactive oxygen and nitrogen species and NF- κ B activation. Interestingly, the paradoxical increase in colorectal cancer rates among post-bariatric surgery patients may be potentially associated with altered bile acid metabolism. While bariatric surgery has a cancer-protective role for certain obesity-related tumors, a large population study performed in Sweden demonstrated that patients who underwent bariatric surgery had an surprising increased risk of CRC, with a standardized incidence ratio of 1.60 (95% CI 1.25–2.02) compared to 1.26 (95% CI 1.14–1.40) in the obese, no surgery cohort, potentially secondary to the anatomic diversion of bile flow that increases the exposure of the colon to bile acids.¹⁶ Other microbe-derived metabolites that are relevant to the development of CRC are the harmful nitrogenous compounds and hydrogen sulfide formed from bacterial protein fermentation, microbial ethanol production leading to the generation of toxic and carcinogenic acetaldehyde, and the release and conversion of phytochemicals into anti-carcinogenic metabolites (see review¹⁶).

Studies using 16S rRNA sequencing have revealed specific patterns associated with CRC. One study demonstrated an increase in rectum-associated bacterial species diversity in patients with colorectal adenomas compared to healthy controls.⁶¹ In addition, Nakatsu *et al.*⁶² compared 16S rRNA sequences from the mucosal microbiome from normal colorectal mucosa, adenomatous polyps, and adenocarcinomas and found distinct alterations across stages of colorectal carcinogenesis in *Fusobacterium*, *Parvimonas*, *Gemella*, *Peptostreptococcus*, *Bacteroides fragilis* and *Leptotrichia* genera enrichment. Similarly, Gao *et al.*⁶³ correlated microbial communities based on 16S rRNA sequencing data to tumor composition and tumor-adjacent tissue in CRC patients. Despite the prevalence of studies detailing these taxonomic differences in the microbiome, the clinical significance of these associations remains to be explored.

Given the association between gut microbial composition and colorectal cancer, creating an early screening tool for CRC based on an individual's unique fecal metagenome biomarkers represents tantalizing translation of microbiome studies. In pursuit of this, Yu *et al.*⁶⁴ performed a metagenomic profiling study of CRC versus control fecal microbiomes in ethnically different cohorts. The authors identified twenty marker genes that distinguished between CRC and control microbiomes in the Chinese group, four of which were also

validated in a Danish cohort of CRC versus control microbiomes. These four microbial biomarkers were further validated in comparison within French and Austrian cohorts, suggesting potential widespread and universal utility for early screening of CRC based on one's microbiome profile

Intestinal Anastomotic Leaks

Intestinal anastomotic leaks are one of the more devastating complications of general surgery. Despite improvements in post-operative medical care and a multitude of studies devoted to the technique or anastomotic configuration associated with the fewest leaks (hand-sewn, stapled, continuous, interrupted, single layer, double-layer, side-to-side, end-to-end, side-to-end, etc.), rates of anastomotic leak have remained largely unchanged over the past 25 years, with reported incidence ranging from 3% to 19%.⁶⁵

Recent investigation into the role of gut microbes in intestinal anastomotic leaks has been pioneered by John C. Alverdy, MD at University of Chicago, and readers are referred to an in-depth review of this topic for full details.¹⁸ The role of the microbiome in anastomotic leaks was first suggested 60 years ago by Cohn *et al.*,⁶⁶ who performed colonic resections with end-to-end anastomoses in dogs and modeled anastomotic leak by ligating all the feeding arterial vessels five centimeters proximal and distal to the anastomoses. They then infused broad-spectrum antibiotics into the anastomoses of half the cohort. In control animals who did not receive antibiotic treatment, devascularized anastomoses led to intestinal perforation that was universally fatal by five days after surgery. At autopsy, each of these animals had a perforation in the necrotic and gangrenous anastomosis, with resulting generalized peritonitis. In stark contrast, in the animals that had received antibiotic treatment, the anastomosed colon was viable despite the devascularization and indistinguishable from non-operated bowel at time of autopsy. A similar study was performed in humans by Schardey *et al.* in the 1990s.⁶⁷ A prospective, randomized, double-blind, placebo-controlled multicenter study compared the rates of esophagojejunal anastomotic leak rates after total gastrectomy between patients who received oral systemic antibiotic decontamination to those who did not. Three percent of patients (n = 3/102) who received antibiotic decontamination experienced an anastomotic leak versus 10.6% (n=11/103) of patients who did not (P=0.05). As such, the authors proposed that local antimicrobial decontamination was a safe and effective measure for the prevention of esophagojejunal anastomotic leakage after total gastrectomy.

More recently, experimental rats were subjected to pre-operative radiation prior to low colonic resection and anastomosis.⁶⁸ Rates of anastomotic leak were compared between irradiated anastomoses that had been inoculated with *Pseudomonas aeruginosa*, a ubiquitous hospital-associated pathogen and colonizer of hospitalized patients, versus irradiated but non-inoculated, control anastomoses. Anastomoses that were inoculated with *P. aeruginosa* had a significant increase in the rate of anastomotic leakage when compared to control, non-inoculated anastomoses (60% vs. 0%, P<0.01). The authors demonstrated that *P. aeruginosa* underwent a genetic mutation to a more invasive and destructive phenotype with enhanced collagenase activity. Blocking this transformation using a modified polyethylene glycol with

a phosphate moiety was enough to prevent the anastomotic leak in irradiated, inoculated tissue.

In another model of anastomotic leak, experimental rats underwent low colonic resection followed by segmental devascularization of the anastomosed area.⁶⁹ The devascularized anastomoses had a 50% increase in the rate of anastomotic leak compared to control anastomoses, but interestingly, the anastomotic breakdown was not due to tissue ischemia secondary to devascularization or hypoxia. Instead, anastomotic tissues that leaked were colonized by strains of *E. faecalis* that had high collagenase activity. Conversely, healed anastomotic tissues were colonized by low collagenase-expressing *E. faecalis*. The authors went on to demonstrate that *E. faecalis* strains with high collagenase activity both degrade collagen I directly and cleave collagen IV indirectly by activating host tissue matrix metalloproteinase 9 (MMP9). The suppression of this collagenase activity, either via eradication of *E. faecalis* with topical antibiotic treatment to the intestine or via pharmacological suppression of MMP9 collagenase activity, was enough to prevent leaks in the devascularized anastomotic tissue. Furthermore, when non-devascularized anastomoses were inoculated with high collagenase *E. faecalis* strains via rectal enemas, the anastomoses exhibited a greater leak rate, mimicking that of the devascularized, leak-prone anastomoses, which was not observed when non-devascularized tissues were inoculated with low-collagenase *E. faecalis*.⁶⁹ Taken together, these studies suggest an intriguing novel mechanism for anastomotic leaks: the attenuated host inflammatory state, secondary to the stress of disease, surgery, and postoperative healing, can transform commensal gut bacteria into a more virulent phenotype, characterized by high collagenase activity and increased invasive and cytotoxic abilities, which in turn, creates more host tissue injury and greater susceptibility to anastomotic leaks.¹⁸

Inflammatory Bowel Disease

Numerous genetic loci have been associated to IBD, a group of disorders consisting of Crohn's disease (CD) and ulcerative colitis (UC), many of which are involved in immune regulation,⁷⁰ but genetic mutations only partly explain the heritability and pathogenesis of disease. Studies exploring the relationship between the gut microbiome and IBD have revealed three patterns: decreased biodiversity, temporal instability, and dysbiosis featuring overrepresentation of potentially pro-inflammatory microbes relative to anti-inflammatory strains.²¹

In a study comparing a 25,000-clone DNA library generated from fecal samples from six patients with CD to a similarly-sized library derived from healthy patients, CD patients demonstrated significantly reduced bacterial complexity versus their healthy counterparts. Sepehri *et al.*⁷² went further and compared both patients with UC and CD to normal controls, and reaffirmed that patients with IBD had reduced microbial diversity and richness versus their healthy counterparts. In addition, the reduced microbial diversity was most prominent within the inflamed tissue of IBD patients compared to adjacent, non-inflamed, tissue. Furthermore, UC patients either in clinical remission or stable maintenance therapy had only 23% stability in their microbiome composition compared to the prior year, whereas

healthy controls had relatively stable microbiomes, with 78% similarity at one year.⁷³ Similar temporal instability was observed in CD patients.⁷⁴

Pathogenic *E. coli*, specifically adherent-invasive *E. coli* (AIEC), is a pro-inflammatory strain disproportionately increased in IBD.⁷⁵ AIEC, as their name suggests, exhibit enhanced ability to adhere to intestinal epithelial cells, and once attached, have increased ability to invade and survive within the host cells when compared to commensal *E. coli* strains. These pathogenic strains of *E. coli* are more prominent in the intestinal mucosa of CD patients than healthy controls, as they were found in the ileal mucosa of 30.4% (n=7/23) of CD patients with chronic lesions versus 6.2% (n=1/16) of ileal mucosal samples from healthy controls. This divergence is likely due in part to CD patients being particularly vulnerable to infection by AIEC. For example, known CD-associated mutations in *NOD2*, *ATG16L1*, and *IRGM* genes impair autophagy (intracellular degradation), a process that would normally limit the survival of AIEC within intestinal epithelial cells.⁷⁶ Epithelial cells of CD patients also exhibit decreased secretion of Paneth cell-associated peptides, which are important contributors to innate immunity and possess anti-microbial functions.⁷⁷ AIEC also have increased affinity for the epithelial cells of CD patients due to associated increased expression of carcinoembryonic antigen-related cell adhesion molecule 6 (CEACAM6), a molecule expressed on the outside of epithelial cells that acts as the receptor for AIEC's pili.⁷⁸ This bacterial pili-host CEACAM6 interaction mediates AIEC adherence to epithelial cells and allows for the invasion of AIEC into host cells. In contrast, AIEC cannot bind to epithelial cells that do not express CEACAM6, such as those found in individuals without IBD. The intracellular growth of AIEC as a result of these CD-associated mutations potentiates the inflammatory response by inducing infected cells to secrete pro-inflammatory cytokines such as TNF-alpha.^{79,80}

Pathogenic dysbiosis has also been identified in UC patients, *e.g.*, high levels of *Fusobacterium varium* within the colonic mucosa.⁸¹ These bacteria are cytotoxic due to an *F. varium*-associated toxin.⁸² A mixture of *F. varium*, when administered into mouse colons via enemas, created UC-like mucosal lesions in the colon, as well as increased inflammatory markers and apoptosis.

Conversely, specific microbes may have a protective effect in IBD and may be under-expressed in the gut microbiome of IBD patients. *Faecalibacterium prausnitzii*, one such anti-inflammatory microbe, is less prevalent in the fecal microbiome of IBD patients.⁸³ *F. prausnitzii* stimulates the release of IL-10, an anti-inflammatory cytokine, and reduces levels of IL-12, a pro-inflammatory cytokine. Oral administration of *F. prausnitzii* is enough to reduce inflammation in a mouse model of colitis.⁸⁴ Furthermore, the probiotic *Lactobacillus casei* has also been shown to decrease the secretion of TNF-alpha, IFN-gamma, and other pro-inflammatory cytokines in cells grown from ileal mucosa samples obtained from CD patients.⁸⁵ SCFA, as previously mentioned, are produced by the bacterial fermentation of fiber, and have also been shown to have an anti-inflammatory effect by promoting the expansion of regulatory T cells.⁸⁶ IBD patients, coincidentally, have fewer high-SCFA producers, such as *Ordoribacter*, *Phascolarctobacterium*, and *Roseburia*.⁸⁷

In summary, there is a distinct IBD microbiome profile characterized by reduced diversity, increased temporal instability, and dysbiosis that favors pro-inflammatory species. However, as with many studies on the human gut microbiome, direct causative links between the gut microbiome and IBD have yet to be demonstrated. Current research is focused on developing these mechanistic links and offer the opportunity to utilize the gut microbiome as both a diagnostic and therapeutic tool for IBD. In-depth reviews of specific host-microbial interactions in IBD are provided by Kostic *et al.*²⁰

Atherosclerosis

An early study of the role of gram-negative organisms in the pathogenesis of atherosclerosis compared aortic root plaque development between atherosclerosis-prone apolipoprotein E knockout mice (*apoE* KO), lipopolysaccharide-resistant (*Lps^d*) mice, and germ-free *apoE* KO mice.⁸⁸ The codominant *Lps^d* allele corresponds to a missense mutation of the gene encoding for Toll-like receptor 4, a transmembrane receptor that is important in recognizing bacteria-associated lipopolysaccharide (LPS) and initiating the innate immune response. *Lps^d* mice demonstrate minimal increases in serum levels of TNF α and IL-6 in response to intraperitoneal injection of LPS. There was no difference in atherogenesis between *apoE* KO and *apoE* KO/*Lps^d* mice at any time point (4–32 weeks of age), and aortic root plaque development (necrotic cores, foam cells, and fibrous caps in the aortic root lesions) was also similar between conventional and germ-free mice, leading the authors to conclude that commensal microbes are not necessary for murine atherogenesis.

However, more recent studies have identified novel mechanistic pathways through which gut microbes could contribute to atherogenesis. Gut microbes metabolize dietary L-carnitine and choline, into trimethylamine, which is metabolized in the liver to trimethylamine-N-oxide (TMAO). In 4,000 patients who underwent elective coronary angiography, fasting levels of TMAO correlated with major adverse cardiovascular events.⁸⁹ In addition, dietary supplementation of *apoE* KO mice with choline, TMAO, or betaine upregulated multiple macrophage scavenger receptors linked to atherosclerosis and promoted aortic plaque development, which was reversed by antibiotic suppression.⁴⁶ Similarly, mice that undergo dietary carnitine supplementation have increased synthesis of TMA and TMAO and increased aortic atherosclerosis.⁹⁰ Finally, transplantation of cecal contents from atherosclerosis-prone mouse strains to atherosclerosis-resistant mouse strains resulted in enhancement of aortic atherogenesis from dietary choline, demonstrating the novel finding that atherosclerosis susceptibility can be transmitted by gut microbial transfer.⁹¹

Alternatively, impaired intestinal permeability and dysbiosis induced by dietary fat may contribute to atherosclerosis and glucose intolerance in a microbe-dependent manner, as demonstrated by increased intestinal permeability, higher circulating endotoxin concentration, and aortic plaque in low-density lipoprotein receptor knockout mice (LDLR KO) fed a Western diet, changes which were ameliorated by oral antibiotics.⁹²

In humans, patients with symptomatic carotid stenosis have altered gut metagenomes compared to age- and sex-matched healthy controls without cardiovascular disease, including enrichment of the genus *Colinsella* and decreased *Roseburia* and *Eubacterium*.⁹³ Patients with advanced atherosclerosis requiring vascular surgery or major amputation also

differed from age- and sex-matched controls without clinically-apparent atherosclerosis in the serum profiles of phenyl and indole microbe-derived metabolites.⁹⁴

CONCLUSION

In summary, this review highlights the impact of the gut microbiome on common surgical diseases and problems, including obesity, NAFLD, CRC, intestinal anastomotic leaks, IBD, and atherosclerosis. Many of the supporting studies demonstrate associations rather than defined mechanistic pathways, and hence fundamental knowledge that further elucidates the cause-effect relationship between gut microbiota and host pathophysiology are eagerly awaited. Furthermore, translations of these relationships to novel **diagnostic and** therapeutic strategies for surgical patients will be equally important as the microbiome medicine field advances.

Acknowledgments

Grant funding: This work was funded in part by T32HL094293 (to E.C. and C.C.); **Abbott Fund (to E.C.)**; K08HL130601 (to K.H.) from the National Heart, Lung, and Blood Institute; American College of Surgeons/ Society of Vascular Surgery (to K.H.); Vascular Cures (to K.H.); and National Institute of Justice award 2017-MU-MU-0042 (to J.A.G.)

References

1. Sender R, Fuchs S, Milo R. Are we really vastly outnumbered? Revisiting the ratio of bacterial to host cells in humans. *Cell*. 2016; 164:337–340. [PubMed: 26824647]
2. Cho I, Blaser MJ. The human microbiome: At the interface of health and disease. *Nat Rev Genet*. 2012; 13:260–270. [PubMed: 22411464]
3. Human Microbiome Project C. Structure, function and diversity of the healthy human microbiome. *Nature*. 2012; 486:207–214. [PubMed: 22699609]
4. Gill SR, Pop M, Deboy RT, Eckburg PB, Turnbaugh PJ, Samuel BS, et al. Metagenomic analysis of the human distal gut microbiome. *Science*. 2006; 312:1355–1359. [PubMed: 16741115]
5. Qin J, Li R, Raes J, Arumugam M, Burgdorf KS, Manichanh C, et al. A human gut microbial gene catalogue established by metagenomic sequencing. *Nature*. 2010; 464:59–65. [PubMed: 20203603]
6. Hutkins RW, Krumbeck JA, Bindels LB, Cani PD, Fahey G Jr, Goh YJ, et al. Prebiotics: Why definitions matter. *Curr Opin Biotechnol*. 2016; 37:1–7. [PubMed: 26431716]
7. Hill C, Guarner F, Reid G, Gibson GR, Merenstein DJ, Pot B, et al. Expert consensus document. The international scientific association for probiotics and prebiotics consensus statement on the scope and appropriate use of the term probiotic. *Nat Rev Gastroenterol Hepatol*. 2014; 11:506–514. [PubMed: 24912386]
8. Fraher MH, O'Toole PW, Quigley EM. Techniques used to characterize the gut microbiota: A guide for the clinician. *Nat Rev Gastroenterol Hepatol*. 2012; 9:312–322. [PubMed: 22450307]
9. Matsen, FAt. Phylogenetics and the human microbiome. *Syst Biol*. 2015; 64:e26–41. [PubMed: 25102857]
10. Sweeney TE, Morton JM. The human gut microbiome: A review of the effect of obesity and surgically induced weight loss. *JAMA Surg*. 2013; 148:563–569. [PubMed: 23571517]
11. Magoulitis DE, Tasiopoulou VS, Sioka E, Chatedaki C, Zacharoulis D. Impact of bariatric surgery on metabolic and gut microbiota profile: A systematic review and meta-analysis. *Obes Surg*. 2017; 27:1345–1357. [PubMed: 28265960]
12. Bashiardes S, Shapiro H, Rozin S, Shibolet O, Elinav E. Non-alcoholic fatty liver and the gut microbiota. *Molecular Metabolism*. 2016; 5:782–794. [PubMed: 27617201]
13. Leung C, Rivera L, Furness JB, Angus PW. The role of the gut microbiota in nafld. *Nat Rev Gastroenterol Hepatol*. 2016; 13:412–425. [PubMed: 27273168]

14. Schnabl B, Brenner DA. Interactions between the intestinal microbiome and liver diseases. *Gastroenterology*. 2014; 146:1513–1524. [PubMed: 24440671]
15. Sears CL, Garrett WS. Microbes, microbiota, and colon cancer. *Cell host & microbe*. 2014; 15:317–328. [PubMed: 24629338]
16. Louis P, Hold GL, Flint HJ. The gut microbiota, bacterial metabolites and colorectal cancer. *Nat Rev Microbiol*. 2014; 12:661–672. [PubMed: 25198138]
17. Gagniere J, Raisch J, Veziant J, Barnich N, Bonnet R, Buc E, et al. Gut microbiota imbalance and colorectal cancer. *World J Gastroenterol*. 2016; 22:501–518. [PubMed: 26811603]
18. Guyton K, Alverdy JC. The gut microbiota and gastrointestinal surgery. *Nat Rev Gastroenterol Hepatol*. 2017; 14:43–54. [PubMed: 27729657]
19. Chassaing B, Darfeuille-Michaud A. The commensal microbiota and enteropathogens in the pathogenesis of inflammatory bowel diseases. *Gastroenterology*. 2011; 140:1720–1728. [PubMed: 21530738]
20. Kostic AD, Xavier RJ, Gevers D. The microbiome in inflammatory bowel disease: Current status and the future ahead. *Gastroenterology*. 2014; 146:1489–1499. [PubMed: 24560869]
21. Manichanh C, Borruel N, Casellas F, Guarner F. The gut microbiota in ibd. *Nat Rev Gastroenterol Hepatol*. 2012; 9:599–608. [PubMed: 22907164]
22. Tang WH, Kitai T, Hazen SL. Gut microbiota in cardiovascular health and disease. *Circ Res*. 2017; 120:1183–1196. [PubMed: 28360349]
23. Ley RE, Bäckhed F, Turnbaugh P, Lozupone CA, Knight RD, Gordon JI. Obesity alters gut microbial ecology. *Proceedings of the National Academy of Sciences of the United States of America*. 2005; 102:11070–11075. [PubMed: 16033867]
24. Turnbaugh PJ, Ley RE, Mahowald MA, Magrini V, Mardis ER, Gordon JI. An obesity-associated gut microbiome with increased capacity for energy harvest. *Nature*. 2006; 444:1027–1031. [PubMed: 17183312]
25. Ley RE, Turnbaugh PJ, Klein S, Gordon JI. Microbial ecology: Human gut microbes associated with obesity. *Nature*. 2006; 444:1022–1023. [PubMed: 17183309]
26. Le Chatelier E, Nielsen T, Qin J, Prifti E, Hildebrand F, Falony G, et al. Richness of human gut microbiome correlates with metabolic markers. *Nature*. 2013; 500:541–546. [PubMed: 23985870]
27. Pallister T, Jackson MA, Martin TC, Glastonbury CA, Jennings A, Beaumont M, et al. Untangling the relationship between diet and visceral fat mass through blood metabolomics and gut microbiome profiling. *Int J Obes (Lond)*. 2017; 41:1106–1113. [PubMed: 28293020]
28. Leone V, Gibbons SM, Martinez K, Hutchison AL, Huang EY, Cham CM, et al. Effects of diurnal variation of gut microbes and high-fat feeding on host circadian clock function and metabolism. *Cell Host Microbe*. 2015; 17:681–689. [PubMed: 25891358]
29. Liu R, Hong J, Xu X, Feng Q, Zhang D, Gu Y, et al. Gut microbiome and serum metabolome alterations in obesity and after weight-loss intervention. *Nat Med*. 2017; 23:859–868. [PubMed: 28628112]
30. Everard A, Belzer C, Geurts L, Ouwerkerk JP, Druart C, Bindels LB, et al. Cross-talk between *akkermansia muciniphila* and intestinal epithelium controls diet-induced obesity. *Proc Natl Acad Sci U S A*. 2013; 110:9066–9071. [PubMed: 23671105]
31. Serino M, Luche E, Gres S, Baylac A, Berge M, Cenac C, et al. Metabolic adaptation to a high-fat diet is associated with a change in the gut microbiota. *Gut*. 2012; 61:543–553. [PubMed: 22110050]
32. Neyrinck AM, Van Hee VF, Bindels LB, De Backer F, Cani PD, Delzenne NM. Polyphenol-rich extract of pomegranate peel alleviates tissue inflammation and hypercholesterolaemia in high-fat diet-induced obese mice: Potential implication of the gut microbiota. *Br J Nutr*. 2013; 109:802–809. [PubMed: 22676910]
33. Anhe FF, Roy D, Pilon G, Dudoone S, Matamoros S, Varin TV, et al. A polyphenol-rich cranberry extract protects from diet-induced obesity, insulin resistance and intestinal inflammation in association with increased *akkermansia* spp. Population in the gut microbiota of mice. *Gut*. 2015; 64:872–883. [PubMed: 25080446]

34. Buchwald H, Estok R, Fahrbach K, Banel D, Sledge I. Trends in mortality in bariatric surgery: A systematic review and meta-analysis. *Surgery*. 2007; 142:621–632. discussion 632–625. [PubMed: 17950357]
35. Liou AP, Paziuk M, Luevano JM Jr, Machineni S, Turnbaugh PJ, Kaplan LM. Conserved shifts in the gut microbiota due to gastric bypass reduce host weight and adiposity. *Sci Transl Med*. 2013; 5:178ra141.
36. Tremaroli V, Karlsson F, Werling M, Stahlman M, Kovatcheva-Datchary P, Olbers T, et al. Roux-en-y gastric bypass and vertical banded gastroplasty induce long-term changes on the human gut microbiome contributing to fat mass regulation. *Cell Metab*. 2015; 22:228–238. [PubMed: 26244932]
37. Furet JP, Kong LC, Tap J, Poitou C, Basdevant A, Bouillot JL, et al. Differential adaptation of human gut microbiota to bariatric surgery-induced weight loss: Links with metabolic and low-grade inflammation markers. *Diabetes*. 2010; 59:3049–3057. [PubMed: 20876719]
38. Zhang H, DiBaise JK, Zuccolo A, Kudrna D, Braidotti M, Yu Y, et al. Human gut microbiota in obesity and after gastric bypass. *Proc Natl Acad Sci U S A*. 2009; 106:2365–2370. [PubMed: 19164560]
39. Samuel BS, Gordon JI. A humanized gnotobiotic mouse model of host-archaeal-bacterial mutualism. *Proceedings of the National Academy of Sciences of the United States of America*. 2006; 103:10011–10016. [PubMed: 16782812]
40. Jahansouz C, Staley C, Bernlohr DA, Sadowsky MJ, Khoruts A, Ikramuddin S. Sleeve gastrectomy drives persistent shifts in the gut microbiome. *Surgery for obesity and related diseases : official journal of the American Society for Bariatric Surgery*. 2017; 13:916–924. [PubMed: 28279578]
41. Rinella ME. Nonalcoholic fatty liver disease: A systematic review. *JAMA*. 2015; 313:2263–2273. [PubMed: 26057287]
42. Dawes EA, Foster SM. The formation of ethanol in *Escherichia coli*. *Biochimica et biophysica acta*. 1956; 22:253–265. [PubMed: 13382840]
43. Zhu L, Baker SS, Gill C, Liu W, Alkhouri R, Baker RD, et al. Characterization of gut microbiomes in nonalcoholic steatohepatitis (NASH) patients: A connection between endogenous alcohol and NASH. *Hepatology*. 2013; 57:601–609. [PubMed: 23055155]
44. Cope K, Risby T, Diehl AM. Increased gastrointestinal ethanol production in obese mice: Implications for fatty liver disease pathogenesis. *Gastroenterology*. 2000; 119:1340–1347. [PubMed: 11054393]
45. Adachi Y, Moore LE, Bradford BU, Gao W, Thurman RG. Antibiotics prevent liver injury in rats following long-term exposure to ethanol. *Gastroenterology*. 1995; 108:218–224. [PubMed: 7806045]
46. Wang Z, Klipfell E, Bennett BJ, Koeth R, Levison BS, Dugar B, et al. Gut flora metabolism of phosphatidylcholine promotes cardiovascular disease. *Nature*. 2011; 472:57–63. [PubMed: 21475195]
47. Kucera O, Cervinkova Z. Experimental models of non-alcoholic fatty liver disease in rats. *World J Gastroenterol*. 2014; 20:8364–8376. [PubMed: 25024595]
48. Dumas M-E, Barton RH, Toye A, Cloarec O, Blancher C, Rothwell A, et al. Metabolic profiling reveals a contribution of gut microbiota to fatty liver phenotype in insulin-resistant mice. *Proceedings of the National Academy of Sciences*. 2006; 103:12511–12516.
49. Peterson LW, Artis D. Intestinal epithelial cells: Regulators of barrier function and immune homeostasis. *Nature reviews Immunology*. 2014; 14:141–153.
50. Gäbele E, Dostert K, Hofmann C, Wiest R, Schölmerich J, Hellerbrand C, et al. DSS induced colitis increases portal LPS levels and enhances hepatic inflammation and fibrogenesis in experimental NASH. *Journal of Hepatology*. 2011; 55:1391–1399. [PubMed: 21703208]
51. Mencin A, Kluwe J, Schwabe RF. Toll-like receptors as targets in chronic liver diseases. *Gut*. 2009; 58:704–720. [PubMed: 19359436]
52. Miele L, Valenza V, La Torre G, Montalto M, Cammarota G, Ricci R, et al. Increased intestinal permeability and tight junction alterations in nonalcoholic fatty liver disease. *Hepatology*. 2009; 49:1877–1887. [PubMed: 19291785]

53. Keusch GT. Opportunistic infections in colon carcinoma. *Am J Clin Nutr.* 1974; 27:1481–1485. [PubMed: 4611196]
54. Waisberg J, de Matheus CO, Pimenta J. Infectious endocarditis from streptococcus bovis associated with colonic carcinoma: Case report and literature review. *Arquivos de gastroenterologia.* 2002; 39:177–180. [PubMed: 12778310]
55. Abdulmir AS, Hafidh RR, Bakar FA. Molecular detection, quantification, and isolation of streptococcus gallolyticus bacteria colonizing colorectal tumors: Inflammation–driven potential of carcinogenesis via il-1, cox-2, and il-8. *Molecular Cancer.* 2010; 9:249. [PubMed: 20846456]
56. Wang X, Allen TD, May RJ, Lightfoot S, Houchen CW, Huycke MM. Enterococcus faecalis induces aneuploidy and tetraploidy in colonic epithelial cells through a bystander effect. *Cancer research.* 2008; 68:9909–9917. [PubMed: 19047172]
57. Soler AP, Miller RD, Laughlin KV, Carp NZ, Klurfeld DM, Mullin JM. Increased tight junctional permeability is associated with the development of colon cancer. *Carcinogenesis.* 1999; 20:1425–1432. [PubMed: 10426787]
58. Vizcaino MI, Crawford JM. The colibactin warhead crosslinks DNA. *Nat Chem.* 2015; 7:411–417. [PubMed: 25901819]
59. Bernstein H, Bernstein C, Payne CM, Dvorak K. Bile acids as endogenous etiologic agents in gastrointestinal cancer. *World J Gastroenterol.* 2009; 15:3329–3340. [PubMed: 19610133]
60. Reddy BS. Types and amount of dietary fat and colon cancer risk: Prevention by omega-3 fatty acid-rich diets. *Environmental Health and Preventive Medicine.* 2002; 7:95–102. [PubMed: 21432290]
61. Sanapareddy N, Legge RM, Jovov B, McCoy A, Burcal L, Araujo-Perez F, et al. Increased rectal microbial richness is associated with the presence of colorectal adenomas in humans. *Isme J.* 2012; 6:1858–1868. [PubMed: 22622349]
62. Nakatsu G, Li X, Zhou H, Sheng J, Wong SH, Wu WK, et al. Gut mucosal microbiome across stages of colorectal carcinogenesis. *Nat Commun.* 2015; 6:8727. [PubMed: 26515465]
63. Gao Z, Guo B, Gao R, Zhu Q, Qin H. Microbiota disbiosis is associated with colorectal cancer. *Front Microbiol.* 2015; 6:20. [PubMed: 25699023]
64. Yu J, Feng Q, Wong SH, Zhang D, Liang QY, Qin Y, et al. Metagenomic analysis of faecal microbiome as a tool towards targeted non-invasive biomarkers for colorectal cancer. *Gut.* 2015
65. Alves A, Panis Y, Trancart D, Regimbeau J-M, Pocard M, Valleur P. Factors associated with clinically significant anastomotic leakage after large bowel resection: Multivariate analysis of 707 patients. *World Journal of Surgery.* 2002; 26:499–502. [PubMed: 11910487]
66. Cohn I Jr, Rives JD. Antibiotic protection of colon anastomoses. *Ann Surg.* 1955; 141:707–717. [PubMed: 14362409]
67. Schardey HM, Joosten U, Finke U, Staubach KH, Schauer R, Heiss A, et al. The prevention of anastomotic leakage after total gastrectomy with local decontamination. A prospective, randomized, double-blind, placebo-controlled multicenter trial. *Annals of Surgery.* 1997; 225:172–180. [PubMed: 9065294]
68. Olivas AD, Shogan BD, Valuckaite V, Zaborin A, Belogortseva N, Musch M, et al. Intestinal tissues induce an snp mutation in pseudomonas aeruginosa that enhances its virulence: Possible role in anastomotic leak. *PloS one.* 2012; 7:e44326. [PubMed: 22952955]
69. Shogan BD, Belogortseva N, Luong PM, Zaborin A, Lax S, Bethel C, et al. Collagen degradation and mmp9 activation by *enterococcus faecalis* contribute to intestinal anastomotic leak. *Science Translational Medicine.* 2015; 7:286ra268–286ra268.
70. de Lange KM, Barrett JC. Understanding inflammatory bowel disease via immunogenetics. *Journal of autoimmunity.* 2015; 64:91–100. [PubMed: 26257098]
71. Ott SJ, Musfeldt M, Wenderoth DF, Hampe J, Brant O, Folsch UR, et al. Reduction in diversity of the colonic mucosa associated bacterial microflora in patients with active inflammatory bowel disease. *Gut.* 2004; 53:685–693. [PubMed: 15082587]
72. Sepehri S, Kotlowski R, Bernstein CN, Krause DO. Microbial diversity of inflamed and noninflamed gut biopsy tissues in inflammatory bowel disease. *Inflammatory bowel diseases.* 2007; 13:675–683. [PubMed: 17262808]

73. Martinez C, Antolin M, Santos J, Torrejon A, Casellas F, Borruel N, et al. Unstable composition of the fecal microbiota in ulcerative colitis during clinical remission. *Am J Gastroenterol*. 2008; 103:643–648. [PubMed: 18341488]
74. Scanlan PD, Shanahan F, O'Mahony C, Marchesi JR. Culture-independent analyses of temporal variation of the dominant fecal microbiota and targeted bacterial subgroups in crohn's disease. *J Clin Microbiol*. 2006; 44:3980–3988. [PubMed: 16988018]
75. Darfeuille-Michaud A, Boudeau J, Bulois P, Neut C, Glasser AL, Barnich N, et al. High prevalence of adherent-invasive *escherichia coli* associated with ileal mucosa in crohn's disease. *Gastroenterology*. 2004; 127:412–421. [PubMed: 15300573]
76. Lapaquette P, Glasser AL, Huett A, Xavier RJ, Darfeuille-Michaud A. Crohn's disease-associated adherent-invasive *e. Coli* are selectively favoured by impaired autophagy to replicate intracellularly. *Cellular microbiology*. 2010; 12:99–113. [PubMed: 19747213]
77. Wehkamp J, Salzman NH, Porter E, Nuding S, Weichenthal M, Petras RE, et al. Reduced paneth cell alpha-defensins in ileal crohn's disease. *Proc Natl Acad Sci U S A*. 2005; 102:18129–18134. [PubMed: 16330776]
78. Barnich N, Carvalho FA, Glasser AL, Darcha C, Jantschkeff P, Allez M, et al. Ceacam6 acts as a receptor for adherent-invasive *e. Coli*, supporting ileal mucosa colonization in crohn disease. *J Clin Invest*. 2007; 117:1566–1574. [PubMed: 17525800]
79. Bringer MA, Glasser AL, Tung CH, Meresse S, Darfeuille-Michaud A. The crohn's disease-associated adherent-invasive *escherichia coli* strain lf82 replicates in mature phagolysosomes within j774 macrophages. *Cellular microbiology*. 2006; 8:471–484. [PubMed: 16469058]
80. Glasser AL, Boudeau J, Barnich N, Perruchot MH, Colombel JF, Darfeuille-Michaud A. Adherent invasive *escherichia coli* strains from patients with crohn's disease survive and replicate within macrophages without inducing host cell death. *Infect Immun*. 2001; 69:5529–5537. [PubMed: 11500426]
81. Ohkusa T, Sato N, Ogihara T, Morita K, Ogawa M, Okayasu I. *Fusobacterium varium* localized in the colonic mucosa of patients with ulcerative colitis stimulates species-specific antibody. *Journal of gastroenterology and hepatology*. 2002; 17:849–853. [PubMed: 12164960]
82. Ohkusa T, Okayasu I, Ogihara T, Morita K, Ogawa M, Sato N. Induction of experimental ulcerative colitis by *fusobacterium varium* isolated from colonic mucosa of patients with ulcerative colitis. *Gut*. 2003; 52:79–83. [PubMed: 12477765]
83. Sokol H, Seksik P, Furet JP, Firmesse O, Nion-Larmurier I, Beaugerie L, et al. Low counts of *faecalibacterium prausnitzii* in colitis microbiota. *Inflammatory bowel diseases*. 2009; 15:1183–1189. [PubMed: 19235886]
84. Sokol H, Pigneur B, Watterlot L, Lakhdari O, Bermudez-Humaran LG, Gratadoux JJ, et al. *Faecalibacterium prausnitzii* is an anti-inflammatory commensal bacterium identified by gut microbiota analysis of crohn disease patients. *Proc Natl Acad Sci U S A*. 2008; 105:16731–16736. [PubMed: 18936492]
85. Llopis M, Antolin M, Carol M, Borruel N, Casellas F, Martinez C, et al. *Lactobacillus casei* downregulates commensals' inflammatory signals in crohn's disease mucosa. *Inflammatory bowel diseases*. 2009; 15:275–283. [PubMed: 18839424]
86. Smith PM, Howitt MR, Panikov N, Michaud M, Gallini CA, Bohlooly YM, et al. The microbial metabolites, short chain fatty acids, regulate colonic treg cell homeostasis. *Science (New York, NY)*. 2013; 341doi: 10.1126/science.1241165
87. Morgan XC, Tickle TL, Sokol H, Gevers D, Devaney KL, Ward DV, et al. Dysfunction of the intestinal microbiome in inflammatory bowel disease and treatment. *Genome biology*. 2012; 13:R79. [PubMed: 23013615]
88. Wright SD, Burton C, Hernandez M, Hassing H, Montenegro J, Mundt S, et al. Infectious agents are not necessary for murine atherogenesis. *The Journal of experimental medicine*. 2000; 191:1437–1442. [PubMed: 10770809]
89. Tang WH, Wang Z, Levison BS, Koeth RA, Britt EB, Fu X, et al. Intestinal microbial metabolism of phosphatidylcholine and cardiovascular risk. *N Engl J Med*. 2013; 368:1575–1584. [PubMed: 23614584]

90. Koeth RA, Wang Z, Levison BS, Buffa JA, Org E, Sheehy BT, et al. Intestinal microbiota metabolism of l-carnitine, a nutrient in red meat, promotes atherosclerosis. *Nat Med.* 2013; 19:576–585. [PubMed: 23563705]
91. Gregory JC, Buffa JA, Org E, Wang Z, Levison BS, Zhu W, et al. Transmission of atherosclerosis susceptibility with gut microbial transplantation. *The Journal of biological chemistry.* 2015; 290:5647–5660. [PubMed: 25550161]
92. Ghosh SS, Bie J, Wang J, Ghosh S. Oral supplementation with non-absorbable antibiotics or curcumin attenuates western diet-induced atherosclerosis and glucose intolerance in *ldlr*^{-/-} mice--role of intestinal permeability and macrophage activation. *PLoS One.* 2014; 9:e108577. [PubMed: 25251395]
93. Karlsson FH, Fak F, Nookaew I, Tremaroli V, Fagerberg B, Petranovic D, et al. Symptomatic atherosclerosis is associated with an altered gut metagenome. *Nat Commun.* 2012; 3:1245. [PubMed: 23212374]
94. Cason CA, Dolan KT, Sharma G, Tao M, Kulkarni R, Helenowski IB, et al. Plasma microbiome-modulated indole- and phenyl-derived metabolites associate with advanced atherosclerosis and postoperative outcomes. *J Vasc Surg.* 2017

Table 1Glossary of microbiome-related terms^{6–9}

Term	Definition
Microbiome	The totality of microbes, their genetic elements (genomes), and environmental interactions within a defined environment
Microbiota	The assemblage of individual microorganisms present in a defined environment. This is a more accurate term than “microflora.”
Taxon	A taxonomic group of any rank, such as a species, family, or class.
Phylogeny	Evolutionary relationships among organisms. Classification of microorganisms based on phylogeny reflect how they evolved over time.
Bacteroidetes and Firmicutes	Major phyla of gut microbes. Changes in the ratio of Firmicutes:Bacteroidetes relative abundance are associated with obesity
Microbial strain	A term commonly used to denote a pure culture, but can also refer to a natural concept closely related to the clone.
16S ribosomal RNA (16S rRNA) gene	The component of the 30S small subunit of the <u>prokaryotic ribosome</u> that binds to the <u>Shine-Dalgarno sequence</u> . The genes coding for it are referred to as the 16S rRNA gene and are conserved between bacterial species but allow for identification of species
Next-generation sequencing	Sequencing technologies that provide large amounts of sequence data rapidly. These technologies include direct sequencing of 16S rRNA gene amplicons using beads (454 Pyrosequencing®), slides (Illumina), and solid surfaces (SOLiD), or “massively parallel sequencing” and shotgun sequencing and metagenomics (see entry below)
Metagenomics	The study of genetic material (<i>e.g.</i> all microbial DNA in a sample) recovered directly from an environmental sample such as the gut. <i>Shotgun metagenomics</i> refers to the process in which the total DNA from a sample is fragmented in a random manner and subjected to next-generation sequencing. This generates primer-independent and unbiased sequencing data which can be analyzed using various reference-based and reference-free methods. Shotgun metagenomics targets all DNA material in a sample and produces relative abundance information for all genes, functions and organisms.
Genome assembly	The genome sequence produced after chromosomes have been fragmented, those fragments have been sequenced, and the resulting sequences have been put back together.
Operational Taxonomic Unit (OTU)	Arbitrarily defined taxonomic unit based on sequence divergence, <i>i.e.</i> , a cluster of similar sequences
Alpha diversity	Diversity within a sample. “Richness” refers to the number of species present in a sample. “Evenness” refers to the relative abundance of different species in the sample. For example, the Chao1 and Shannon diversity metrics both measure richness and evenness.
Beta diversity	Diversity (distance or dissimilarity) between samples. Principal Coordinate Analysis (PCoA) plots are 2 and 3-dimensional plots that visualize data in beta diversity distance matrices. A weighted UniFrac beta diversity metric accounts for abundance of organisms and phylogeny information. An unweighted UniFrac beta diversity metric accounts only for presence or absence of organism and phylogeny information.
Metabolomics	A study of the collective metabolites present in a sample
Metaproteomics	A study of the collective proteins present in a cell, tissue, or organism or expressed by a genome
Metatranscriptomics	A study of the collective mRNA present in a sample
Germ-free mouse	Mice that are born and raised without any microorganisms, including bacteria, fungi, and viruses.
Prebiotic	Nutrients that promote the growth or activity of microorganisms
Probiotic	Live microorganisms that have a beneficial effect when consumed

Table 2

Surgical diseases that have been linked to specific changes in gut microbial composition

Disease or condition	Selected gut microbes or microbiome changes associated with disease	Mechanisms linking dysbiosis to pathogenesis	Selected references
Obesity	Relative decrease in Bacteroidetes and concomitant increase in Firmicutes prevalence in obesity Increased prevalence of methanogenic (methane-producing) archaea in obese individuals	Obesity-associated microbes have increased capacity for energy harvest Methanogens control the fermentation of dietary fiber by consuming hydrogen and acetate during methanogenesis, which drives the fermentative process by removing these secondary byproducts, making fermentation of fiber more energetically favorable for those bacteria capable of the process	10,11
NAFLD / NASH cirrhosis		Increased intestinal permeability exposes liver to pro-inflammatory factors such as lipopolysaccharide from gram-negative organisms Microbial production of ethanol leads to increased intrahepatic triglyceride accumulation and generation of reactive oxygen species Microbial hydrolysis of choline decreases host capacity for VLDL production, leading to increased intrahepatic deposition of triglycerides and steatosis Bile acids produced by microbial metabolism promotes lipid oxidation and reduces inflammation and fibrosis, namely through the bile acid activated Farnesoid X Receptor (FXR) Microbial fermentation of carbohydrates generates short-chain fatty acids (SCFA), which, as an energy source for the liver, help regulate hepatic lipogenesis and gluconeogenesis	12–14
Colon cancer	<i>S. gallolyticus</i> <i>E. faecalis</i> , <i>E. coli</i> <i>B. Fragilis</i>	Colonization is associated with increased IL-1 and COX2 gene expression, potentiating a chronic inflammatory state in colon Produce superoxide radicals leading to DNA damage Secrete <i>B. fragilis</i> toxin, which cleaves and deactivates E-cadherin, a known tumor suppressor Microbes deconjugate primary bile acids into pro-inflammatory and carcinogenic secondary bile acids Bacterial protein fermentation leads to oncogenic nitrogenous compounds and hydrogen sulfide Microbial production of ethanol, as well as subsequent microbial metabolism of, leads to increased carcinogenic acetaldehyde production. Generation of SCFAs that have been shown to be anti- carcinogenic and induce apoptosis of colorectal cancer cells Microbial biotransformation of protective phytochemicals and xenobiotics	15–17
Intestinal anastomotic leaks	<i>P. aeruginosa</i> and <i>E. faecilis</i>)	Bacteria degrade collagen directly or indirectly by the activation of host tissue matrix metalloproteinases	18
Inflammatory bowel disease	Decreased biodiversity		19–21

Disease or condition	Selected gut microbes or microbiome changes associated with disease	Mechanisms linking dysbiosis to pathogenesis	Selected references
	<p>Increased fluctuation of microbial composition over time</p> <p>Adherent-invasive <i>E. coli</i></p> <p><i>Fusobacterium varium</i></p> <p><i>Faecalibacterium prausnitzii</i>, <i>Lactobacillus casei</i>, <i>Ordoribacter</i>, <i>Phascolarctobacterium</i>, <i>Roseburia</i></p>	<p>Preference for Crohn's disease host cells due to decreased intracellular defense (lack of autophagy) and increased binding affinity (expression of CEACAM 6 on host cells). Invasion of AIEC generates a pro- inflammatory cascade</p> <p>Produces pro-inflammatory <i>F. varium</i> associated toxin</p> <p>Associated with reduced expression in IBD patients and attenuate inflammation</p>	
Atherosclerosis		Microbial metabolism of dietary L-carnitine and choline leads to increased trimethylamine-N-oxide (TMAO). Levels of TMAO correlate with major adverse cardiovascular events	22