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The Human Microbiome and Surgical Disease

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Abstract

Objective—The purpose of this review article is to summarize what is currently known about microbes associated with the human body and to provide examples of how this knowledge impacts the care of surgical patients.

Background—Pioneering research over the past decade has demonstrated that human beings live in close, constant contact with dynamic communities of microbial organisms. This new reality has wide-ranging implications for the care of surgical patients.

Methods and Results—Recent advances in the culture-independent study of the human microbiome are reviewed. To illustrate the translational relevance of these studies to surgical disease, we discuss in detail what is known about the role of microbes in the pathogenesis of obesity, gastrointestinal malignancies, Crohn disease, and perioperative complications including surgical site infections and sepsis. The topics of mechanical bowel preparation and perioperative antibiotics are also discussed.

Conclusions—Heightened understanding of the microbiome in coming years will likely offer opportunities to refine the prevention and treatment of a wide variety of surgical conditions.

Pioneering research over the past decade has demonstrated that human beings live in close, constant contact with vast communities of microbial organisms. This new reality has wide-ranging implications for the care of surgical patients.¹ It has been estimated that the number of microbes in or on the human body outnumbers the total number of human cells by a factor of ten.² Collectively, these organisms make important contributions to our physiology during periods of both health and illness. For this reason, experts in the field have proposed that human beings are most appropriately characterized as “superorganisms” composed of both human and microbial cells.³ Viewed in this fashion, it is clear that many traditional aspects of medical and surgical thinking must be recalibrated. The purpose of this review

article is to summarize what is currently known about microbes associated with the human body and to provide examples of how this knowledge impacts the care of surgical patients.

THE CULTURE-INDEPENDENT REVOLUTION

It is generally agreed that the vast majority of microbial species on the planet have not yet been successfully isolated or cultivated in the laboratory.⁴ In the gastrointestinal tract, which harbors the majority of microbes associated with the human body, it has been estimated that between 10% and 50% of organisms can be successfully grown in clinical microbiology laboratories.⁵ The inability to cultivate most organisms reflects inadequate knowledge of their nutritional and environmental requirements.⁶ The stark reality that most of the microbial world is so poorly characterized has obvious implications for clinicians that routinely rely upon culture results to care for their patients.

In the past two decades, novel molecular approaches have made it possible to study organisms in detail without cultivating them in the laboratory. These advances have revolutionized our ability to identify which organisms are present within a biologic sample, and also to understand the functions encoded by the genomes of these organisms. Culture-independent ecologic surveys of microbial diversity (Figure 1) most commonly rely upon amplification and sequencing of genes encoding the 16S ribosomal subunit.⁷ The 16S gene, present within all microbial cells, is highly conserved except within its so-called hypervariable regions. Nucleotide sequences in these variable regions are very similar in organisms from the same species, but they are divergent among organisms from distinct species.⁸ A number of investigations have utilized 16S-based methods to determine the identities of organisms present in biological samples in both experimental and clinical settings. These have ranged from routine investigations of the gut flora^{9,10} to esoteric studies of microbial communities on biliary stents¹¹ or the tips of bladder catheters after prostatectomy.¹² The use of molecular techniques that do not rely upon cultivation greatly enhances the sensitivity and accuracy of such studies.

During this microbial renaissance, the number of organisms with completed genomes has grown rapidly. The first bacterial genome to be fully sequenced was *Haemophilus influenzae* in 1995,¹³ 6 years before a draft of the human genome was released.¹⁴ Since that time, over 1000 microbial genomes have been finished.¹³ These efforts have made it possible to know a great deal about the functional potential of an individual bacterial species. From a medical standpoint, this creates opportunities to understand the molecular basis of microbe-mediated surgical diseases. Serving as an example of the translational importance of advances in microbial pathogenomics, a remarkable series of recent reports has established that single nucleotide changes in the genome of group A *Streptococcus* can dramatically impact whether or not an at-risk patient will develop necrotizing fasciitis.¹⁵

It has also become clear that a surprising degree of genomic variation can exist between two bacterial cells from the same species. Studies of *Escherichia coli* and *Streptococcus* have demonstrated that only 75% of a microbial genome is required for the organism to live and reproduce, whereas up to 25% of a genome can be specific to a particular strain or variant.^{16,17} This 25% of “flexible” genes often impacts the pathogenicity of a given

organism, and routine culture-based techniques are generally not sensitive enough to evaluate the presence or expression of such genes.¹⁸ The age-old task of trying to determine whether organisms such as coagulase-negative staphylococci or *Candida albicans* are present as colonizers or pathogens on surgical wards will be greatly aided by ongoing attempts to determine the genetic sequences of such organisms.

THE HUMAN MICROBIOME

The “human microbiome” is defined as the collective set of genomes of the microbes associated with the human body.¹⁹ These microbes include species of bacteria and archaea, and a number of viruses that can infect humans directly or that can infect other microbes. Owing to robust advances in high-throughput sequencing technologies, it is now possible to study the microbiome on a grand scale that was not possible just years ago. Accordingly, the NIH has established the Human Microbiome Project (HMP) as a Roadmap Initiative.²⁰ With the support of the HMP, a great deal of attention has been turned toward defining which microbes are “normal” and which may be associated with disease pathogenesis. The translational importance of microbes is relevant to research within every NIH institute, and certainly to every surgical discipline.

At least three important properties of the human microbiome are now well characterized. First, as described earlier, is the complexity of the microbiome. The average human body harbors 10^{14} microbial cells. The vast majority of these cells are located in the gastrointestinal tract, but nontrivial bacterial communities are also associated with the genitourinary tract, the skin, and the oral cavity.²⁰ Second, spatial distribution of microbes within the body is nonrandom. It has been shown that host factors including temperature, pH, and level of moisture each contribute to the formation of distinct ecological niches within the body.⁶ It is not surprising that resident microbes of the stomach differ from those of the colon. But clinicians may find it more surprising to learn that distinct sets of organisms are found even in different microenvironments within the oral cavity,²¹ or that microbial diversity on the palmar surface of the hand is strongly affected by handedness and sex of the person being studied.²² Third, although clinicians tend to view bacteria negatively,³ microbes associated with the human body can be beneficial, neutral, or pathogenic. Largely in the context of studies of germ-free animals, it has been shown that the “normal flora” is actually necessary for proper postnatal growth and development.²³ In a striking recent example of the importance of the indigenous flora, it was demonstrated that commensal staphylococcal species are indeed required for normal epithelial inflammation and wound healing.²⁴ As discussed below, clinicians should be aware that indiscriminate use of antibiotics affects the growth and function of the commensal flora as well as the growth of pathogens.

The microbes of the skin and the gastrointestinal tract (2 anatomic regions with obvious relevance to surgeons) have been well characterized with culture-independent techniques in the past few years. Both of these regions of the human body possess unique structural and physiologic characteristics that enable them to interact with the outside microbial world and to simultaneously protect underlying organs from infection. Because of the dry and acidic microenvironment, most organisms are unable to colonize the surface of the skin or hair

follicles.⁶ A study recently published with support from the HMP demonstrated that nearly all cutaneous bacteria can be assigned to 1 of 4 bacterial supergroups or phyla: Actinobacteria (51.8%), Firmicutes (24.4%), Proteobacteria (16.5%), and Bacteroides (6.3%).²⁵ The bacterial genera observed most frequently in this study were *Corynebacterium* (22.8%; from the phylum Actinobacteria), *Propionibacterium* (23%; Actinobacteria), and *Staphylococcus* (16.8%; Firmicutes). The authors of this study demonstrated that microbial communities vary according to skin location and type. Sebaceous regions of the skin (eg, the upper chest or back) are dominated by propionibacteria and staphylococci. Moist microenvironments, eg within the inguinal or gluteal creases, are dominated by corynebacteria. Dry regions such as the forearm or the buttock contain relatively mixed populations of bacteria with a relatively high abundance of beta-proteobacteria such as *Neisseria*. The anterior nares of most individuals studied did harbor *Staphylococcus aureus*, whereas other regions of the body generally did not.

The relationship between the microbiome and different regions of the skin is similar to the variations observed in the distinct compartments of the gastrointestinal tract. In general, because food passes so rapidly, microbial communities do not establish in the lumens of the upper GI tract.⁶ A limited number of organisms successfully colonize the mucosa of the upper tract, for example, *Helicobacter pylori* within the hostile environment of the stomach. By contrast, the ileum, cecum, colon, and rectum harbor large and complex microbial communities. It has been elegantly demonstrated that vital functions of the gut microbiota include conversion of otherwise indigestible food components, production of essential cofactors and vitamins, and stimulation of the innate immune system.^{23,26} The anaerobic environment of the colon is dominated by the phyla Firmicutes and Bacteroidetes; species such as *Bacteroides fragilis*, *Bacteroides thetaiotamicron*, and the clostridial species predominate.²⁶ The well-known family Enterobacteriaceae (that contains medically relevant genera such as *Escherichia*, *Klebsiella*, *Pseudomonas*, and *Salmonella*) actually represents a tiny fraction of the microbial community in the distal intestine; facultative anaerobic organisms such as these probably amount to less than 1% of the colonic flora.

WHY THE HUMAN MICROBIOME PROJECT IS RELEVANT TO SURGEONS

A broad range of medical and surgical problems have been linked to perturbations of the microbiome. Several topics are discussed below in detail, and additional topics that have been investigated with molecular studies of the microbiome are listed in Table 1.^{27–38}

Obesity

Evidence continues to emerge demonstrating the critical role that gut bacteria play in regulating host energy metabolism and body mass index. For example, gut flora make significant contributions to the degradation of nondigestible foodstuffs such as plant polysaccharides and fiber present in our diet. Sequencing the genomes of gut bacterial species (eg, *Bacteroides thetaiotamicron*) has revealed the presence of enzymatic capacities inherent to bacteria that are absent in the human genome. The presence of these enzymes allows for bacterial digestion of nutrients that would be otherwise inaccessible to the host.³⁹ In addition to regulating polysaccharide utilization, gut bacteria have also been found to

impact fat storage. It has been shown that normal gut flora may stimulate increased lipoprotein lipase activity and triglyceride storage by inhibiting Fiaf (fasting-induced adipocyte factor), a protein inhibitor of lipoprotein lipase.⁴⁰ It has been proposed that alterations in some gut bacterial communities may thus limit the ability of lipoprotein lipase to adequately process triglycerides, thereby promoting the development of obesity.

Several groups have sought to demonstrate a causal link between gut bacteria and host obesity. Recent work has demonstrated that transfer of cecal microflora from obese mice to lean mice promotes increased weight gain in the lean mice.^{41,42} Investigations of germ-free mice have similarly demonstrated the importance of microbes in the development of obesity. For example, germ-free mice raised in aseptic isolation are leaner than conventionally raised mice *despite increased food intake*.^{43,44} The germ-free mice actually require a 30% increase in caloric intake to maintain the same weight as their conventionally raised counterparts.⁴⁵ In addition, within 14 days of transfer of microbiota from conventionally raised obese mice to germ-free lean mice, germ-free lean mice develop up to a 60% increase in body fat.⁴³ Further supporting the key role of bacteria in obesity, it has been shown that mice lacking Toll-like Receptor 4, a cell surface receptor that recognizes bacteria, are resistant to diet induced obesity.⁴⁶

Ongoing attempts have been made to identify specific bacterial species that may promote obesity. Multiple groups have demonstrated that, relative to controls, Bacteroidetes species are less abundant and Firmicutes species are more abundant in rodent models of obesity.⁴² The Firmicutes rich microbiome is thought to promote obesity by increasing the supply of enzymes that break down dietary polysaccharides and promoting nutrient absorption.⁴² Transcriptional profiling of microbes in the cecal contents of lean and obese mice has documented an increased expression of dietary energy extraction genes in obese mice.⁴²

The presence of an altered gut microbiome in the setting of obesity is not isolated to mice; support for this finding has also been demonstrated in rats,^{47,48} pigs,⁴⁹ and humans.^{50,51} For example, in humans provided with either a fat-restricted or carbohydrate-restricted diet, the relative proportion of Bacteroidetes species to Firmicutes species increased over time along with reductions in host body weight.⁵⁰ Also, in an analysis of fecal samples from twins who were discordant for obesity or leanness, the obese state was found to be associated with an overall reduction in bacterial diversity, a decreased abundance of Bacteroidetes species, and increased expression of genes associated with carbohydrate and lipid metabolism.⁵² Importantly for surgeons, patients who underwent a Roux-en-Y gastric bypass were found to have a decreased abundance of Firmicutes bacteria in the intestine as compared to their obese counterparts.^{41,53}

Given the strong evidence that the gut microbiome is directly linked to obesity, it is conceivable that manipulation of the gut flora could elicit weight loss in obese patients. Presumably, if health care providers could nonsurgically alter the gut microbiome to create a bacterial phenotype consistent with decreased energy harvest, novel medical therapies for obesity could be developed. For example, given the association of Firmicutes bacteria with obesity, selective antibiotic administration against species in this phylum may be a feasible approach to confer weight loss. Alternatively, given the findings of the bacterial transfer

experiments in germ-free mice, it is reasonable to suggest that transfer of bacterial species from nonobese to obese individuals could similarly promote weight loss in human patients. These are examples of nonsurgical options that may eventually be available in the treatment of obesity.

Cancer

In contrast to viruses (eg, hepatitis B and the human immunodeficiency virus), bacteria have not historically been associated with carcinogenesis; however, it is likely that increased attention to the human microbiome will uncover links between cancer and our commensal flora. The most relevant and best-studied example of an association between carcinogenesis and bacterial colonization is *Helicobacter pylori*. Convincing evidence has linked *H. pylori* infection with both gastric adenocarcinoma^{54,55} and mucosa-associated lymphoid tissue (MALT) lymphoma,^{56,57} leading to its classification as a class I carcinogen by the World Health Organization. Infection with *H. pylori*, a Gram-negative urease-producing bacterium, affords a 2- to 20-fold increase in the risk of developing gastric adenocarcinoma.

Upon colonization of the gastric mucosa in some individuals, *H. pylori* elicits both inflammatory and immune responses through the release of cytotoxic compounds, leading to the development of peptic ulcer disease and chronic atrophic gastritis.⁵⁸ The extent of gastritis and intestinal metaplasia is directly related to the risk of developing adenocarcinoma. The persistent inflammation that ensues after infection has several deleterious biological consequences, including accumulation of reactive oxygen and nitrogen species, both of which are mutagenic and can directly cause cellular damage.⁵⁹ In addition, chronic inflammation leads to increased gastric epithelial cell proliferation, which in turn increases the risk for DNA replication error.⁶⁰ Although infection with *H. pylori* is strongly associated with the development of gastric cancer, only 0.1% to 0.5% of people infected with the bacteria go on to develop adenocarcinoma.⁶⁰ More work is needed to determine which infected patients are at highest risk for developing carcinoma.

It has become clear that *H. pylori*-induced carcinogenesis represents a complex relationship between bacterial determinants of virulence and a range of host genetic factors. For example, one of the most widely studied virulence determinant of *H. pylori* is the cytotoxin-associated gene A (*cagA*). Bacterial strains carrying the *cagA* gene are strongly associated with an increased risk of developing atrophic gastritis, peptic ulcer disease, and gastric adenocarcinoma.^{61–64} The *CagA* protein is delivered into gastric epithelial cells by the bacterial type IV secretion system, where it interrupts intracellular signaling pathways.⁶⁴ Two subtypes of the *cagA* gene exist: the East Asian type and the Western type.⁶⁵ Clinically, the severity of atrophic gastritis and the risk of gastric malignancy are significantly higher in patients with East Asian *cagA* positive strains. Importantly, humans can carry multiple strains of *H. pylori*, and isolates within an individual can change over time as chromosomal rearrangements, mutations, and recombination events occur.⁶⁶ Heterogeneity within bacterial populations likely contributes to variability in clinical manifestations.

Colorectal cancer continues to be a significant cause of cancer-related death in the United States. The observation that the risk of developing colorectal cancer varies markedly between and within populations and geographical regions has led to the hypothesis that

specific dietary practices can be a risk factor for disease.^{67,68} In fact, the geographical variation in colon cancer incidence can be quite dramatic, with westernized countries significantly more affected than developing countries. Several studies have linked the large consumption of meat in westernized countries with up to a 17% increased risk of colorectal cancer.⁶⁹ One explanation of how diet impacts cancer risk is through the presence of nonabsorbable carcinogens from environmental contaminants or created during food preparation. A second and more complex explanation involves the interactions between the host microbiota and unabsorbed products that enter the colon; indeed, there is great interest at present in how gut bacteria may contribute to the diet-induced risk of colorectal cancer.

Disturbances in the gut microbial community can generate toxins that promote chronic inflammation and mutagenic compounds, both of which are known to increase the risk of cancer. An example of this concept can be seen in populations that consume large amounts of sulfur-rich meats. Ingestion of large quantities of dietary sulfur leads to the overgrowth of sulfur-reducing bacteria (eg, *Desulfovibrio vulgaris*), resulting in increased production of the highly toxic compound hydrogen sulphide.⁷⁰ It is known that hydrogen sulphide impairs cytochrome oxidase, suppresses the vital utilization of butyrate, generates toxic free radicals, and inhibits mucus synthesis.^{70,71} Investigators in England have demonstrated that up to 70% of fecal samples obtained from study subjects who ate typical westernized diets (ie, high in meat) contained increased concentrations of hydrogen sulphide. By contrast, low concentrations of hydrogen sulphide were observed in rural South African subjects, a population known to have lower rates of meat consumption and a significantly lower incidence of colorectal cancer.^{70,71}

Another example of how cancer risk is mediated by diet and gut bacteria involves the metabolism of high-fat diets. The consumption of fat stimulates the synthesis of cholic acid, a primary bile acid, of which a small portion typically reaches the colon.⁷² If the colon contains 7-alpha-dehydroxylating bacterial colonies, which are more abundant in westernized countries, cholic acid is converted to the well-recognized carcinogen deoxycholic acid.⁷³ All of these studies taken together illustrate how diet can affect colonic health and cancer risk by impacting the host microbiota. The enormous complexity of the colonic ecosystem makes it extremely challenging to perform high-resolution studies of how individual species or dietary components impact the risk of developing cancer. Nonetheless, it is clear that further examination of interactions between diet and the microbiome may contribute to an improved understanding of the pathogenesis of colorectal cancer.

CROHN DISEASE

Recent research has also helped elucidate links between gut bacteria and Crohn disease (CD). In broad terms, investigators have pursued 3 hypotheses that may explain how bacteria contribute to CD pathogenesis. First, it remains possible that a specific causative pathogen is responsible for the disease. Second, it may be that CD results not from one pathogen but rather from a generalized disordering of the normal gut microbial community as seen in obesity. Third, several groups have evaluated whether immune dysfunction and changes in tolerance yield an inappropriate autoimmune response to commensal organisms. None of these hypotheses have yet been proved.

Two pathogens have drawn particular attention as putative causative agents in CD: *Mycobacterium avium* subspecies paratuberculosis (MAP) and adherent/invasive *Escherichia coli*. *E. coli* (AIEC) has been isolated from the mucosa of patients with CD more commonly than in healthy controls, but MAP has been much more thoroughly investigated as a potential pathogen.⁷⁴ MAP has repeatedly been documented in the blood and tissues of patients with CD.^{75,76} However, there is a high amount of variability in the percentage of samples harboring MAP (between 0% and 100%).^{12,77} In the case of AIEC, it has been shown that these bacteria selectively colonize the macrophages and epithelial cells of the ileum in patients with Crohn disease.⁷⁸ In fact the prototypic AIEC strain LF82 is able to induce granulomas in an in vitro model using blood-derived mononuclear cells.⁷⁹ However, the theory that these (or other) infectious agents directly cause CD is greatly weakened by the fact that immunosuppression does not exacerbate CD, that there are no studies demonstrating transmissibility of disease, and the fact that antibiotic administration has not provided a sustained benefit.^{80,81}

At present, there is great enthusiasm for the dysbiosis theory of CD pathogenesis. This theory suggests that there is an altered balance between beneficial and aggressive bacterial species in patients with CD.⁸² There is evidence that an alteration of bacteria in both the intestinal lumen and the mucus layer may play a role.⁸³ Multiple studies have shown that the fecal microflora differs between CD patients and healthy controls.^{84–86} In general, it has been found that there is a decreased complexity of the commensal intestinal flora in CD patients.⁸⁴ Specifically, CD patients have been demonstrated to possess increased fecal concentrations of *Bacteroides vulgatus* and decreased amounts of lactobacilli and bifidobacteria.⁸⁷ In addition it has been found that patients with CD have higher antibody titers against *Escherichia coli* than controls.⁸⁸ Crohn disease patients have also exhibited increased risk of recurrence after resection when they were found to have low preoperative levels of the bacteria *Faecalibacterium prausnitzii*, a member of the phylum Firmicutes.⁸⁹ Beyond the differences found in the fecal stream of patients with CD there may also be differences in the quantity of bacteria residing in the intestinal mucus layer. It has been suggested that patients with CD have a higher number of bacteria in the mucus layer than healthy controls.⁹⁰

Patients with CD seem to be defective in clearing both normal and abnormal species of gut bacteria; functionally, this represents a breakdown in normal mechanisms of tolerance to the commensal flora. It has been proposed that failure of first-line mechanisms to clear bacteria in CD is followed by compensatory antibacterial immune responses involving T cells that can cause tissue damage. An important series of articles has documented the association between CD and polymorphisms in the nucleotide-binding oligomerization domain NOD 2 (also known as CARD15). NOD2/CARD15 functions as an intracellular receptor for muramyl dipeptide (MDP), a component of both Gram-negative and Gram-positive bacterial cell walls.⁹¹ Binding of MDP to NOD2/CARD15 leads to the activation of a pro-inflammatory pathway mediated by NFκB.⁹² NOD2/CARD15 polymorphisms in CD are associated with ileal involvement, younger age at onset of CD, and fibrostenosing disease.⁹³ Additional genetic polymorphisms have been identified in 2 components of the autophagy pathway (ATGL16L1 and IRGM) that influences the ability of humans to kill intracellular bacteria.⁹⁴ These “upstream” defects may lead to downstream overgrowth and translocation

of intestinal bacteria and excessive stimulation of T cells and the pathognomonic granulomatous inflammation seen in CD.

Administering probiotics to manipulate the pathogenicity of the bacterial flora is a promising medical therapy for CD, although trials evaluating the efficacy of this approach have thus far been underpowered and met with mixed success. Probiotics are defined as dietary supplements of live microorganisms with putative health benefits that are derived from the human intestinal tract.⁹⁵ Oral administration of *Lactobacillus acidophilus*, *Bifidobacterium lactis*, and *Lactobacillus casei* has been shown to prevent intestinal inflammation in experimental models of colitis.⁹⁶ *L. casei*, in particular, has demonstrated an ability to act as an anti-inflammatory mediator by decreasing the secretion of inflammatory mediators including tumor necrosis factor, IFN- γ , IL-2, and IL-6 from explants of CD mucosa.⁹⁷ Efforts have been made to translate these laboratory results into improved clinical outcomes. A recent meta-analysis determined that probiotics did not prevent postoperative recurrence of CD.⁹⁸ However, it is likely that further studies, sufficiently powered, will be conducted to better characterize the effects of probiotics on this and other diseases.

THE MICROBIOME IN THE ICU

The striking variability in the clinical course of infected patients highlights the complexity of host-microbe interactions. As noted, human beings and their colonizing microbes generally maintain a well-tolerated symbiotic relationship. However, colonization by potential pathogens can dramatically alter the consequences of physiologic perturbations after surgical stress. Similarly, interindividual variability in the host inflammatory response to colonizing microbes contributes to the manifestation and course of surgical infection. Variability in the host inflammatory response is influenced by many factors including genetic predisposition,⁹⁹ the nature and extent of surgical stress, the presence of comorbid conditions, and effects of pharmacologic therapies (eg, immunosuppressive drugs, antimicrobials, or vasopressors).

Host-microbe interactions occur continuously at interfaces such as the skin, respiratory tract, and GI tract. However, physiologic changes associated with surgical stress (eg, tissue hypoxia, hypophosphatemia, iron sequestration, and so on) can dramatically transform the microenvironment of the host-pathogen interface.¹⁰⁰ The fact that most surgical patients do not experience infectious complications after surgery highlights the potent plasticity and adaptability of both the host and microbe in response to surgical stress. However, in specific patients, disordering of the host-microbe relationship can yield heightened virulence expression by bacteria and a fulminant inflammatory response in the host.^{101,102} In this scenario, the host-microbe relationship may no longer be mutually beneficial. At present, there is little understanding of how genetic and environmental variables lead to infectious complications in some patients but not others. Improving the diagnosis and treatment of surgical infections will require simultaneous molecular investigations of patients, their microbiomes, and the dynamic interactions between them.

With the molecular techniques referred to above, we are beginning to obtain fundamental information about which microbes are present¹⁰³ in clinical settings. However, while it is

important to document which organisms are present, it is critical to also acknowledge that regulation of bacterial gene expression in response to environmental signals directs the temporal and spatial variation in virulence expression. Microbes are constantly assessing and reassessing their local microenvironment and altering phenotypic expression to optimize their fitness and survival.¹⁰⁴ In contrast to the traditional understanding of virulence as a constant trait, expression of virulence is tightly regulated and selectively expressed in response to specific environmental cues.¹⁰⁵ Uncovering these environmental “triggers” is a formidable challenge as microbes are exposed to a wide array of potential signals in the clinical setting. In the relatively short time that the translational importance of virulence regulation has been appreciated, several stress-related cues have been identified by our laboratory and others including nutrient scarcity (phosphate,¹⁰⁶ iron¹⁰⁷), products of ischemia reperfusion injury (adenosine¹⁰⁸), host immune mediators (interferon¹⁰⁹), and the neurohormonal stress response (endogenous opioids¹¹⁰).

Ultimately, insights into the regulation and expression of microbial virulence offer the potential to transform our approach to the treatment of surgical infections. At present, surgical infections (surgical site infections, anastomotic leaks, intraabdominal abscesses, gut-derived sepsis) are treated with local control when possible (debridement, drainage) and antibiotic therapy. The strategy of microbial elimination may itself be misguided as the indiscriminant elimination of commensal microbes with antimicrobial therapy can precipitate nontrivial secondary infections and/or antibiotic resistance. Emerging virulence-based strategies capitalize on the context-dependent nature of virulence expression with the goal of pacifying, as opposed to eliminating, offending pathogens.¹¹¹ By targeting the “sense and response” mechanisms of virulence expression, this approach may preserve the contributions of commensal flora to host health and circumvent some problems inherent to conventional antimicrobial therapies. For the surgeon, continued development of minimally invasive techniques to reduce operative stress and reduce host inflammatory responses may also attenuate triggers for virulence expression and reduce infectious complications.^{112,113}

PERIOPERATIVE CONSIDERATIONS

As we gain more knowledge about microbes associated with our patients, we are learning that many routine aspects of surgical care can impact the state of the microbiome and therefore can impact clinical outcomes. The use of mechanical bowel preps (MBP) in elective colorectal operations is a practice that has come under close scrutiny in the last several years. Surgical site infections and anastomotic leakage after these procedures have been associated with mortality rates of up to 6% and 5% to 19%, respectively.^{114,115,116} The putative advantages of the routine use of MBP are not only to prevent infectious complications but also to make the colon easier to manipulate for the surgeon. However, recent studies underscore the potentially deleterious effects of MBP.

The intestinal mucosal layer is a dynamic cellular barrier that is influenced by host factors and its constituent flora. Commensal microorganisms in this environment act to suppress opportunistic pathogens and to enhance the mucosal barrier function.⁹ Although overall intraluminal and intramucosal colony counts are unaffected by MBP, it seems that MBP may act to upset the normal ecology of this space by altering the endogenous flora and providing

an opportunity for colonization by potentially opportunistic pathogens.^{117–119} Furthermore, it has been demonstrated that increased intraluminal pressure caused by such an osmotic load alters intracellular signaling pathways, cell proliferation, and cell matrix interactions, all of which play a role in the gut barrier function.^{120,121} The continued use of MBP must be carefully considered, given that multiple, carefully designed randomized controlled trials have demonstrated no difference in outcomes between prepped and unprepped patients.^{122,123} Furthermore, some meta-analyses have indicated that the incidence of serious complications such as surgical site infection and anastomotic dehiscence is increased in individuals receiving MBP.^{124,125}

The use of perioperative antibiotics is considered to be standard of care in selected circumstances, and the proper timing, regimen and duration of therapy are viewed as important quality measures by national entities such as the Surgical Care Improvement Project (SCIP).¹²⁶ The rationale behind the use of such drugs is to reduce bacterial burden and to effect a decrease in the rate of surgical site infections, a problem that costs an estimated \$1.5 billion a year.¹²⁷ However, the use of prophylactic antibiotics should be carefully evaluated because inappropriate overuse leads to the emergence of resistant organisms and because inadequate antibiotic use may adversely affect patient outcomes. This has been shown to be true in patients suffering from pneumonia, bacteremia, and most recently surgical site infections as measured by endpoints ranging from length of stay to death.^{128–130} We must strike a fine balance between undertreating and overtreating infections. Work in animal models has demonstrated that broad-spectrum antibiotic therapy results in long-lasting (but not permanent) alterations in intestinal colonization profiles⁹ and an increased susceptibility to colonization by pathogenic organisms such as *Salmonella enterica*.¹³¹ The importance of these ideas is, of course, highlighted by the incidence and morbidity associated with the unchecked growth of *Clostridium difficile* in patients treated with broad-spectrum antibiotics.¹³²

It may be possible and indeed desirable to catalogue the dominant bacterial species associated with patients admitted to the hospital. This personalized information could become clinically relevant by helping to predict which patients might be at highest risk for complications such as *C. difficile* infection. A recent report from the Netherlands demonstrated that the incidence of surgical site infections could be reduced by rapidly screening and decolonizing carriers of *S. aureus* on admission to the hospital.¹³³ These investigators used a simple real-time polymerase chain reaction assay followed by mupirocin nasal ointment and chlorhexidine soap in those individuals found to be carriers. These interventions collectively yielded a significant reduction in the risk of hospital-acquired *S. aureus* infections in at-risk patients and a significant reduction in mean hospital stay. With this fascinating result in mind, it is easy to envision a future where presurgical evaluations include a culture-independent evaluation of a patient's microbiome. This could serve not only to tailor appropriate therapy against specific pathologic organisms, but also to provide insight into the dynamic interactions between the endogenous flora and clinical outcomes.¹³⁴

CONCLUSIONS

This review has summarized clinical aspects of recent research related to the Human Microbiome Project. As knowledge of the microbiome has blossomed, it has become abundantly clear that enormous opportunities are now on hand to better understand the pathogenesis of diseases that involve microbes. Novel therapies are certain to follow. These are welcome developments in light of the rising incidence of microbe-mediated diseases and the growing prevalence of drug-resistant organisms. The relevance of this work cannot be understated but has not yet received widespread attention in the surgical community. Moving forward, exciting collaborative efforts between clinicians, microbiologists, and bioinformatics specialists are likely to positively impact the care of patients with surgical disease.

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FIGURE 1.
Schematic representation of culture-independent analysis of bacteria within surgical specimens.

TABLE 1

Partial List of Culture-Independent Studies of Bacteria in Clinical Specimens From Patients With Surgical Disorders

Surgical Specialty	Topic(s)	Reference
Cardiothoracic surgery	Empyema	27
	Prosthetic valve endocarditis	28
Foregut surgery	Barrett's esophagus	29
Hepatobiliary surgery	Cholecystitis	30
Colorectal surgery	Pouchitis after proctocolectomy	31
	Colonic adenomas	32
Vascular surgery	Atherosclerotic plaques	33
	Aortic aneurysm	34
Wound care	Nonhealing foot ulcers	35
	Venous stasis ulcers	36
Transplantation	Intestinal microbiome after small bowel transplantation	37
	Diabetes	38