Published in final edited form as:
Surg Clin North Am. 2011 August ; 91(4): 771–785. doi:10.1016/j.suc.2011.05.001.

Contributions of Intestinal Bacteria to Nutrition and Metabolism in the Critically Ill

Michael J. Morowitz, MD[a], **Erica Carlisle, MD**[b], and **John C. Alverdy, MD**[c]

[a] Assistant Professor of Surgery, Division of Pediatric General and Thoracic Surgery, University of Pittsburgh School of Medicine

[b] Surgical Research Fellow, Resident in General Surgery, University of Chicago Pritzker School of Medicine

[c] Professor of Surgery, University of Chicago Pritzker School of Medicine

SYNOPSIS

Important advances in the study of bacteria associated with the human gastrointestinal tract have significant implications for clinicians striving to meet the metabolic and nutritional needs of critically ill patients. A transition from culture-based to culture-independent studies of the intestinal microbiota has ushered in a new era of laboratory and clinical studies in this field. These studies are helping to clarify the important role of bacteria in carbohydrate metabolism, and are providing new evidence that highlights the role of bacteria in protein and lipid homeostasis. We know that during periods of caloric excess or deprivation, microbial populations in the GI tract are clearly altered; however the molecular etiology for such changes remains elusive. Similarly, little is known about how microbial ecology changes before, during, and after critical illness. Nevertheless, several approaches, e.g. probiotic administration, have been employed to manipulate gut microbial communities in the ICU. In this review we offer a broad overview of the importance of the host-microbe relationship, discuss what is currently known about the role of gut microbes in nutrition and metabolism in the healthy human host, review how gut microbes are impacted by critical illness, and discuss interventions that have already been utilized to manipulate the gut microbiome in ICU patients.

Keywords

gut bacteria; microbiome; nutrition; obesity; critical care

[b]Corresponding author for proof and reprints: John C. Alverdy, MD, jalverdy@surgery.bsd.uchicago.edu, Sarah and Harold Lincoln Thompson Professor, Executive Vice Chair, Department of Surgery, University of Chicago Pritzker School of Medicine, University of Chicago Medical Center, 5841 S. Maryland Avenue, MC 6090, Chicago, IL 60637, Phone 773.702.4876, Fax 773.834.0201.
[a]Coauthor address: Michael J. Morowitz, MD, michael.morowitz@chp.edu, Division of Pediatric General and Thoracic Surgery, Children's Hospital of Pittsburgh of UPMC, Faculty Pavilion 7th Floor, 4401 Penn Avenue, Pittsburgh, PA 15224, Phone 412.692.5976, Fax 412.692.8299

The authors have nothing to disclose.

MICROBES AND NUTRITION DURING CRITICAL ILLNESS

It has been known for decades that intestinal bacteria make important contributions to human metabolism and physiology. Perhaps the example best known to clinicians is the microbial synthesis of the essential nutrient vitamin B12 — the enzymes required for B12 synthesis are possessed by bacteria but not by plants or animals [1]. However, research from the past decade has conclusively established that the host-microbe relationship in humans is far more complex than previously appreciated. The implications of this research for assessing and meeting the nutritional needs of critically ill patients are substantial.

The goals of this review are: (i) to offer a broad overview of the importance of the host-microbe relationship, (ii) to detail what is known about the host-microbe relationship with regard to nutrition and metabolism in the healthy host, (iii) to review the scarce existing literature about how microbial ecology changes during critical illness, and (iv) to discuss specific interventions that have been used to manipulate the gut flora to improve patient nutrition and outcomes in the intensive care unit (ICU).

REVOLUTIONARY ADVANCES IN UNDERSTANDING THE HUMAN MICROBIOME

An understanding of the complex relationship between humans and our microbes dates back at least to Pasteur. However, until very recently, the ability of microbiologists and clinicians to characterize and dissect this relationship was hampered by the reality that only a minority of microbes on the planet (and in the human body) can be cultured, isolated, and systematically studied in the laboratory [2]. As a result, most clinical focus on bacteria and viruses has been directed toward the statistical minority of organisms that cause clinical disease and can be easily isolated in culture.

Over 25 years ago, microbial ecologists conclusively demonstrated that bacterial DNA can be used to identify which organisms are present in a complex biological sample without dependence on cultivating those organisms in the laboratory [3]. Until recently, these culture-independent techniques to characterize microbial diversity were relatively restricted to studies of ocean and soil samples. Over the past decade, concerted efforts have been made to use these techniques to undertake comprehensive molecular surveys of the organisms associated with humans. These efforts have benefited from remarkable advances in DNA sequencing technologies, as well as from well-funded initiatives such as the NIH Human Microbiome Project [4, 5] and its European counterpart MetaHIT [6].

Perhaps the foremost lesson of these recent efforts has been that all humans, both healthy and critically ill, are intimately associated with a vast population of microbial organisms. Uncertainty remains regarding the precise number of bacteria in the human body, but it is generally agreed that there at least 10 bacterial cells for every 1 human cell [7]. This has led authorities in the field to describe humans as “superorganisms” composed of both human and microbial cells [8]. Although clinicians have not historically thought about their patients in this way, it is easy to recognize the evolutionary logic of a symbiotic relationship between humans and microbes. By supporting lifelong colonization by organisms that possess a diverse set of metabolic capabilities, the host effectively augments its own genome; this is a much more efficient arrangement than waiting for humans to evolve new metabolic capacities on their own [9]. In return for their contributions, microbes associated with the body are rewarded with a relatively safe, predictable, and nutrient-rich niche for colonization. As will be discussed in subsequent sections, the impact of critical illness on this symbiotic relationship remains poorly understood.

All epithelial surfaces that interface with the external world harbor microbes, but the most dense microbial communities are those in the distal intestinal tract. Recent estimates suggest that 10 to 100 trillion microbes (including up to 1000 species) reside in this location [8, 10]. Remarkably, although more than 70 bacterial divisions (deep evolutionary lineages) are known to exist on the planet, human gut microbial communities are dominated by just four lineages. Two dominant divisions, the Bacteroidetes and the Firmicutes, comprise over 95% of the total community; most of these organisms are strict anaerobes such as the *Bacteroides* and *Clostridium* species [11]. The remainder of human gut microbes are often from two other divisions: Actinobacteria (e.g., *Bifidobacterium* species) and Proteobacteria. The phylum Proteobacteria contains the gram-negative enterics that despite being well known to clinicians, represent only a fraction of the gut microbial community [11]. The dominance of these four bacterial phyla and the relative absence of all other phyla suggests that, under normal circumstances, the human-microbe relationship is highly selective and highly stable. Throughout most of a person's life, this relationship is either symbiotic (mutually beneficial) or commensal (providing benefit to one member without harming the other); pathogenic host-microbe interactions are indeed the exception rather than the rule [9].

There is currently enormous interest in characterizing the clinical relevance of the human microbiome (defined as the collective set of microbial genomes associated with the human body). In addition to the GI tract, important sites of colonization also under study include the skin, oropharynx, respiratory tract, and genitourinary tract. A primary objective of current research is to better define the basic features of the human microbiome, e.g., how do microbial communities change over time in a given individual and how much interindividual variability is observed in various microbial communities? An equally important objective is to identify associations between the microbiome and human health and disease [12].

SPECIFIC CONTRIBUTIONS OF THE GUT MICROBIOTA TO HUMAN METABOLISM

A particularly compelling example of the importance of the gut microbiota to host metabolism is provided by comparing the nutritional status of germ-free (GF) and conventionally raised laboratory animals. Numerous investigators have demonstrated that conventionally raised animals require up to 30% less caloric intake to maintain their body weight [9]. This remarkable observation is not only surprising; it is also counterintuitive since one might reasonably expect that bacteria and their human host may *compete* for a limited supply of ingested nutrients. In this section, we summarize what is known about how microbes directly impact human nutrition.

Microbiota and carbohydrates

The sophisticated relationship that has evolved between the human GI tract and gut microbiota allows for efficient utilization of dietary carbohydrates. In the proximal GI tract, simple sugars such as glucose are absorbed, and disaccharides (e.g., lactose) are hydrolyzed into their corresponding monosaccharide components such that they too can be absorbed [9]. However, a significant portion of dietary carbohydrates, including complex plant-derived polysaccharides and unhydrolyzed starch, normally passes undigested through to the distal GI tract [13]. Here, dense microbial populations (up to 10^{11} cells per gram of colonic matter) are present that are well-equipped to hydrolyze complex carbohydrates. Many of the enzymes required to utilize these dietary substrates are not encoded in the human genome; by contrast, the microbiome, which contains approximately 100x more genes than the human genome, is highly enriched in such enzymes [9].

Utilization of complex polysaccharides via fermentation by anaerobic bacteria in the large intestine results in the accumulation of short chain fatty acids (SCFA) [14]. The principal SCFAs seen in the colon, acetate, propionate, and butyrate, have inherent nutritional value, but also impact gut epithelial physiology in other ways. They are absorbed by passive diffusion across the colonic epithelium, and are subsequently utilized by different organs. Acetate, the SCFA produced in highest concentration, is used by skeletal and cardiac muscle and can be used by adipocytes for lipogenesis. Butyrate is metabolized primarily in the gut epithelium to yield ketone bodies or CO2 [9]. Interestingly, the colonic epithelium derives up to 70% of its energy needs directly from butyrate. Propionate metabolism is poorly understood but appears to involve transport to the liver by the portal circulation. It is believed that SCFAs also impact water absorption, local blood flow, and epithelial proliferation in the large intestine [9].

Genomic analysis of gut bacteria offers vivid examples of the role of microbes in nutrient utilization. For example, in 2003, Xu, et al. published the complete genome sequence of the gram-negative anaerobe *Bacteroides thetaiotaomicron*, a prominent member of the normal intestinal microbiota [10]. Annotation and analysis of the genome revealed a sophisticated apparatus for acquiring and digesting otherwise unusable dietary polysaccharides. This apparatus, including a complex, multi-component, multi-enzyme complex starch utilization system (SUS), consists of over 230 glycoside hydrolase and 15 polysaccharide lyase genes [15]. The genomic analysis demonstrated that *B. thetaiotaomicron* has evolved the remarkable capacity to sense the availability of carbohydrates in its microenvironment, and that it also has the ability to forage and utilize host-derived glycans (e.g., mucin and heparin). Mechanistic studies in gnotobiotic animals further demonstrated that, when *B. thetaiotaomicron* senses a scarcity of fucose in the intestinal lumen, it actually induces the gut epithelium to upregulate expression of fucosylated glycans that can be used by the bacteria as an energy source without harming the host [16]. This body of work illustrates how the remarkable host-microbe symbiosis can be teased apart by pairing genomic sequencing efforts with creative *in vivo* laboratory studies.

Microbiota and protein metabolism

In contrast to carbohydrates, relatively little attention has been paid to the relationship between the intestinal microbiota and nitrogen balance in humans. This is partly because conventional wisdom states that all essential amino acid requirements in humans must be supplied by the diet [17]; however, emerging evidence indicates that gut microbes can impact nitrogen balance by *de novo* synthesis of amino acids and intestinal urea recycling. These contributions are most pronounced in ruminant animals that, amazingly, can live on a protein-free diet because their microbiota is capable of synthesizing most or all amino acids required for survival.

Microbial synthesis of essential amino acids has been notoriously difficult to measure in humans, but studies with radiolabelled tracers, e.g., ^{13}C and ^{15}N, indicate that the intestinal microbiota makes a measurable contribution to the pool of essential amino acids. A series of experiments involving labeled inorganic nitrogen suggests that up to 20% of circulating lysine and threonine in nonruminant mammals, including adult humans, is synthesized by gut microbes [18, 19]. Similarly, Raj, et al. demonstrated that gut microbial synthesis of leucine in adult men was approximately 20% of the dietary amount [17]. Interestingly, another study demonstrated that several substrates required for microbial synthesis of essential amino acids are derived from dietary carbohydrates [20]. Taken together, these studies provide compelling evidence that gut microbes contribute to the circulating pool of essential amino acids. More work is needed to define these contributions in both healthy and undernourished humans.

The intestinal microbiota also contributes to nitrogen balance by participating in urea nitrogen salvaging (UNS) [21, 22]. Elevated urease expression in gut microbes results in metabolism of urea in the GI tract into ammonia and carbon dioxide. Some of the ammonia can be utilized for microbial synthesis of amino acids. Perhaps more importantly, the nitrogen generated during this process (urea nitrogen) can re-enter the host circulation and serve as a substrate for synthetic processes [23]. Interestingly, reduced urea recycling has been reported in GF animals [24] and in humans receiving antibiotic therapy [25]. Furthermore, several reports indicate that regulation of UNS is important in settings of low N intake and high N demand (e.g., during pregnancy and during periods of rapid somatic growth in infancy) [26–28]. While still relatively preliminary, these studies underscore the relationship between gut microbes and protein metabolism that will likely be further described through on-going characterization of the human microbiome.

Microbiota and lipid metabolism

Until recently, few studies of the association between lipid metabolism and the microbiome existed. However, important research by Jeffrey Gordon, Fredrick Backhed, and colleagues suggests that the body's supply of triglycerides, a prominent source of energy during critical illness [29], is tightly linked to the intestinal microbiota. These findings have enormous potential relevance for research in a wide range of disease states, including metabolic disorders such as obesity (see below) and cardiovascular disease.

This line of inquiry began with comparisons of lipid metabolism in GF and conventionally-raised adult mice. By use of x-ray absorptiometry and epididymal fat pad weight analysis, it was demonstrated that wild-type (WT) animals contained 42% more total body fat than GF animals, *despite a higher metabolic rate* and *a reduced daily consumption of standard chow* [30]. To mechanistically evaluate this finding, the authors transferred the microbiota of WT animals to GF animals. A rapid increase (within 10 days) of total body fat content and epididymal fat weight was noted despite no significant difference in total body weight. Intriguingly, colonization of GF mice with just a single gut microbe (*B. thetaiotaocmicron*, discussed above) also yielded a significant increase in total body fat content, although the increase in fat content was less than that seen with transfer of the complete mouse microbiota. Further work in this model suggested that the microbiota stimulates increased hepatic triglyceride production and promotes storage of adipocyte triglycerides by suppressing the activity of a circulating inhibitor of lipoprotein lipase [30].

These pioneering studies have led to a sustained effort to understand the relationship between the microbiota and adiposity. In one interesting experiment, GF mice were colonized with gut bacteria from humans fed with a typical Western diet (high fat, high carbohydrate), and a similar increase in adiposity was seen in the GF mice [31]. Other experiments that analyzed the lipids present in the serum and adipose tissue of WT and GF mice show that WT animals had elevated levels of 18 phosphatidylcholine species and decreased levels of nine triglyceride species relative to GF animals [32]. Alternatively, in the adipose tissue the concentration of most phosphatidylcholine compounds was similar between the two groups, but an increased concentration of triglycerides was detected in WT animals. Even more between group differences were detected in the liver lipid profiles. For example, in addition to numerous differences in cholesteryl ester and phosphatidylcholine species, WT mice had a significant increase in 95 types of liver triglycerides. The translational relevance of these findings must still be defined, but these results provide clues to the role of microbes in lipid metabolism.

Vitamins

Most human diets provide a robust supply of vitamins, the essential human nutrients that must be obtained from exogenous sources. However, it has long been recognized that gut microbes also contribute to vitamin synthesis. The magnitude of this contribution in healthy and unhealthy patients is currently poorly understood.

It has been known for nearly a century that ruminants have no dietary requirement for water-soluble vitamins as a consequence of the dense microbial populations in the rumen, and that GF laboratory animals require dietary supplements of vitamins that are not needed by their WT counterparts [33]. Several bacterial genera that are common in the distal intestine (e.g., *Bacteroides*, *Bifidobacterium*, and *Enterococcus*) are known to synthesize vitamins. Thiamine, folate, biotin, riboflavin, and panthothenic acid are water-soluble vitamins that are plentiful in the diet, but that are also synthesized by gut bacteria. Likewise, it has been estimated that up to half of the daily Vitamin K requirement is provided by gut bacteria [33]. Interestingly, the molecular structure of bacterially synthesized vitamins is not always identical to the dietary forms of the vitamins. In fact, several specialized epithelial transporters have been recognized to participate specifically in the absorption of vitamins derived from gut bacteria [34]. Perhaps the relative ease in replenishing vitamin stores in ICU patients has minimized enthusiasm for aggressive investigation of how bacterial vitamin biosynthesis is altered in hospitalized patients.

LESSONS LEARNED FROM STUDIES OF NUTRIENT EXCESS AND DEPRIVATION

Studying the relationship between the gut microbiota and energy balance in the extreme states of obesity and starvation may improve our ability to assess and satisfy nutritional needs in the ICU.

Obesity

Studies of energy balance in conventional and GF animals led to the hypothesis that the microbial ecology of the GI tract contributes to the pathogenesis of obesity [35]. Although it is widely acknowledged that excessive caloric intake is the root cause of obesity, it is reasonable to question whether an individual's metabolic response to caloric excess might vary according to the gut microbiota. Much of the work in this area has relied upon a rodent model of obesity in which animals homozygous for a mutation in the leptin gene (*ob*/*ob*) harbor a fully penetrant obese phenotype [36]. Early studies utilizing 16S ribosomal RNA based genetic sequencing identified that obese animals have a markedly decreased abundance of Bacteroidetes organisms (such as *B. thetaiotaomicron*) and a corresponding increase in Firmicutes [36]. Obese mice also possessed an abundance of methanogenic organisms from the domain Archaea, and it is believed that these organisms can aid in bacterial fermentation in the gut via removal of H_2 [37]. The microbial differences observed in these experiments were division wide, i.e., not skewed by the presence or absence of a single species. Further, the differences could not be explained by differences in food consumption. Of central importance, corresponding studies have shown similar features of the gut microbes in obese humans [38, 39].

Why would a microbial community enriched in Firmicutes promote obesity? Recent work has suggested that the microbiota of obese individuals has an increased capacity to harvest energy from the diet [35]. Landmark papers, utilizing high-throughput metagenomic sequencing platforms to identify as many genes as possible from all members of a mixed population of bacteria, from Gordon, Turnbaugh, Ley and colleagues, have conclusively demonstrated that the metabolic potential of the gut microbiome varies according to the

microbial community composition. Molecular analysis of the microbiota of lean and obese mice demonstrated that the obese microbiome is markedly enriched in genes enabling breakdown of dietary polysaccharides, e.g., glucosidases, galactosidases, and amylases, and genes encoding proteins that transport and metabolize the products of polysaccharide metabolism [37]. Biochemical and bomb calorimetry analyses in the same experiments demonstrated increased concentrations of SCFA's (indicating a higher degree of bacterial fermentation) and significantly less energy remaining in the feces of obese mice relative to their lean counterparts [37]. Finally, these phenotypic traits were transmissible; colonization of GF animals with the microbiota of obese animals led to higher weight gain than colonization with microbiota from lean WT mice.

Turnbaugh, et al. have advanced these ideas even further by demonstrating that the microbiome associated with diet-induced obesity (DIO) (in contrast to the ob/ob mutant model) is also rich in Firmicutes species and is similarly efficient at extracting energy from the diet [31]. This set of experiments utilized a mouse model of DIO in which conversion to a high fat/high sugar (Western) diet reliably produces increased total body weight and increased epididymal fat content. The authors demonstrated that DIO alters gut microbial ecology by supporting the growth of Firmicutes species, and, in this case, they detected a specific association between obesity and the abundance of a class of organisms (Mollicutes) from the Firmicutes division that has also been identified in humans. Transplantation of cecal contents from DIO mice, similar to experiments with the ob/ob mice, yielded higher increases in body weight and fat than when cecal contents were transplanted from lean, WT animals. Here, again, metagenomic analyses were used to prove that the gut microbiome of animals fed a Western diet is enriched in genes encoding proteins related to energy harvest, including phosphotransferase proteins that enable the transport of simple sugars such as glucose and fructose.

A critical lesson from this body of work is that alterations in the microbiome of obese individuals are reversible. Early on, Ley, et al. demonstrated that the ratio of Firmicutes to Bacteroidetes species decreases over time in humans on either a fat-restricted or carbohydrate-restricted diet [39]. This was subsequently supported by Turnbaugh's findings that the bloom in Mollicutes seen in DIO was reversible with dietary manipulation [31]. Additional studies monitoring changes in the microbiota after surgical and non-surgical weight loss interventions have produced similar findings [40–42].

Fasting

Because caloric excess and obesity are associated with an altered gut microbiota, a corollary hypothesis is that the mirror-image pattern of alterations would be observed during periods of nutrient deprivation. This question is central to the issue of whether the host-microbe relationship might be exploited to improve the nutritional status of critically ill patients. Surprisingly, relatively little is known about the impact of short and long term fasting on the gut microbiota.

In 1968, Tennant, et al. demonstrated that GF mice do not survive as long as WT mice during starvation despite similar patterns of starvation-induced weight loss. However, this group did not characterize the microbiota of the WT animals [43]. In 1974, Tannock and Savage used a culture-based approach to characterize the intestinal bacteria of mice exposed to a stress model that included deprivation of food, water, and bedding for 48 hours[44]. They concluded that stressed animals had a reduction in *Lactobacilli* and total mucosal-associated bacteria relative to control animals, but maintained a similar number of colonic anaerobes. In 1989, Deitch, et al. similarly reported that starvation induced a decrease in *Lactobacilli* in the murine GI tract, however they noted a bloom of gram-negative enteric organisms. Subsequently, several studies have contrasted gut microbes in newborn animals

receiving either enteral or parenteral nutrition. These studies suggest that TPN-fed animals have an increased relative abundance of potential pathogens, such as *Clostridium perfringens*, that can forage on glycans lining the gut epithelium [45, 46]. However, it is not known if these findings can be extended to critically ill adults that have shifted abruptly from the fed to the fasting state.

Two recent studies harnessed the power of high-throughput DNA sequencing to profile changes in microbial ecology during fasting in animal models. Crawford, et al. performed a fascinating study of myocardial ketone body metabolism by the intestinal microbiota during nutrient deprivation [47]. After a 24 hour fast, the authors observed a significant increase in the abundance of Bacteroidetes species and a corresponding decrease in Firmicutes; this is the converse of what was observed in models of caloric excess. They proceeded to provide convincing evidence that the microbiota plays an integral role in fasting-induced hepatic ketogenesis, an important energy source during stress and starvation. In GF animals, ketogenesis was markedly reduced, and it was shown that myocardial metabolism was redirected towards glucose utilization. To understand further how microbial ecology is altered during fasting, Costello, et al. performed an innovative study in which they studied the Burmese python, a vertebrate that consumes large meals between long intervals of fasting [48]. These authors also demonstrated an abundance of Bacteroidetes during fasting that shifted towards a post-prandial abundance of Firmicutes. Species that were enriched in the post-prandial state included *Clostridium* and *Lactobacillus*. These innovative studies serve as a foundation to study gut microbes in hospitalized patients that are not candidates for enteral nutrition.

WHAT HAPPENS TO THE MICROBIOME DURING CRITICAL ILLNESS?

High-throughput culture-independent techniques have not yet been widely applied to study how the human microbiome changes during critical illness. However, several clinical trials have evaluated strategies to manipulate the gut flora without thoroughly assessing the microbiome before or after therapy. Given the emerging evidence that the microbiota contributes to normal physiology, it stands to reason that therapeutic attempts to eradicate pathogens might be coupled with attempts to restore the “normal” microbiota. For example, the above discussion suggests that optimizing the balance between Bacteroidetes and Firmicutes is a promising, but untested, strategy to improve energy balance among the critically ill.

To date, evaluations of the microbial ecology of the ICU have largely been restricted to culture-based studies. Not surprisingly, studies frequently demonstrate that patients admitted to the ICU are rapidly colonized with opportunistic pathogens [49–52]. It has also been shown that pathogens detected by routine surveillance of the airways or the GI tract can serve as harbingers of an ensuing clinical infection by that organism [53, 54]. Frequently encountered organisms in skin, oropharyngeal, endotracheal, and fecal samples from critically ill patients include the gram-negative enterics as well as species of *Candida*, *Pseudomonas*, and *Staphylococcus*. However, it is critical to emphasize that the fate of commensal organisms, many of which serve beneficial purposes, in the ICU is poorly understood. For this reason, a trial with prospective monitoring of the microbiome in ICU patients with comprehensive culture-independent techniques is needed.

Although we lack a comprehensive molecular readout of gut microbes in the ICU, several human and animal studies provide clues about how the microbiota is altered by common ICU exposures. Several excellent studies have demonstrated that the pervasive, site-specific, and drug-specific effects of antibiotic therapy on the microbiota can be long-lasting [55–57]. Multiple host factors relevant to the critically ill, including epithelial inflammation and

hypoxia, are also known to perturb the microbiota and encourage the overgrowth of pathogens [58, 59]. Some of the most commonly used pharmaceutical agents in the ICU, including acid suppression therapies, vasopressors, and opioids, are known to impact the human microbiota [60, 61]. Finally, our group was the first to demonstrate that the use of total parenteral nutrition or enteral nutrition with processed liquid diets dramatically alters the intestinal microbiota such that bacterial translocation to extraintestinal sites is promoted. As the effects of artificial nutrition, polypharmacy, and the selective pressures of extreme physiologic stress and injury accumulate over the course of critical illness, their impact on the ecologic health of the intestinal microbiota is likely to have a major untoward effect on recovery. Clinical interventions that can preserve gut microbial communities such that a benefit in overall recovery is realized will require more in-depth analysis of the direct impact of these interventions on the gut flora.

SELECTIVE MANIPULATION OF THE GUT MICROBIOTA IN THE ICU

If one accepts that a “healthy” intestinal microbiota serves important biological functions, then it is reasonable to hypothesize that gut microbial communities can be manipulated or “optimized” during critical illness to increase the chances of achieving desired clinical outcomes. In theory, manipulation of the gut microbiota could be used to improve energy balance and decrease the incidence of infectious complications. A fundamental problem with clinical application of this theory has been that we lack a detailed understanding of if and how the microbiome is altered during critical illness. As a result, interventions in this field have been introduced with a limited scientific foundation. Nonetheless, several strategies to optimize the microbiome have now been evaluated clinically. Some, such as the recent description of fecal transplantation for *Clostridium difficile* colitis [62], will not be discussed here. Others with obvious relevance to nutrition are discussed.

Gut decontamination

Over the past two decades, several clinical trials have documented that selective decontamination of the gastrointestinal tract and/or the oropharynx improves outcomes in critically ill patients while simultaneously promoting the growth of antibiotic resistant bacteria [63]. Accepted approaches to decontamination consist of administering a regimen of broad-spectrum nonabsorbable antibiotics that theoretically spares the colonic anaerobes, and instead targets yeast, gram-negative pathogens (e.g., the *Enterobacteriaceae* and *Pseudomonas aeruginosa*), and gram-positive pathogens (e.g., *Staphylococcus aureus*) in the oral cavity or the GI tract. These protocols drastically alter the ICU microbiota [63], and by extension decrease both mortality and the incidence of infectious complications such as ventilator-associated pneumonia [51, 64, 65]. Importantly, although these landmark studies serve as proof of principle that the intestinal microbiota can be manipulated in the ICU to achieve desirable outcomes, no studies utilized molecular techniques to profile the ICU microbiome before, during, or after decontamination. As a result, a precise understanding of how decontamination protocols work is lacking. Nevertheless, enthusiasm for decontamination protocols has diminished due to unacceptable increases in drug-resistant bacterial strains within the ICU.

Probiotics

The administration of probiotics and prebiotics represents an increasingly popular alternative to gut decontamination protocols. Probiotics are defined as live microorganisms that confer health benefits upon humans and animals that ingest them in adequate amounts [66]; prebiotics are nondigestible food ingredients that confer health benefits by selectively inducing the growth of probiotic species [67]. Commonly, probiotics and prebiotics are administered together as a food or dietary supplement known as a synbiotic [67]. Although

trials in a wide range of clinical settings have demonstrated great promise regarding the safety and efficacy of these supplements [67]), many critical issues pertaining to their usage remain unresolved. Interestingly, despite the fact that they are often used to treat patients with disease, probiotics and prebiotics are viewed by regulatory agencies as nutritional supplements rather than as pharmaceutical agents or biohazards. This definition has allowed for lax oversight in the field which has resulted in the commercial use of the terms probiotics and prebiotics even when scientific criteria for the terms have not been met [67].

The practice of administering live microbes with putative health benefits to unhealthy patients dates back to the early twentieth century. Much of the early work in the field was performed at the Pasteur Institute in Paris, where Nobel laureate Eli Metchnikoff and others advanced the notion of a differential gut microbiota in health and disease [68]. These scientists hypothesized that the protective effects of specific diets in some regions of Europe could be attributed to the diet-induced growth of beneficial microbes. Interestingly, this led almost instantly to commercial attempts to capitalize upon these ideas, hence the development of probiotics. The most commonly used probiotic species are nonpathogenic yeasts and organisms from the genera *Lactobacillus* and *Bifidobacterium* [69]. The most commonly used prebiotics are the naturally occurring oligosaccharides known as fructans that are normally found in foods such as garlic, artichokes, and bananas [67]. Another well-studied class of prebiotics is resistant starches, such as those found in unripe bananas and raw potatoes. As knowledge of the intestinal microbiome expands, it is likely that many more potential probiotic species and prebiotic supplements will be identified.

The long list of clinical diagnoses that have been treated with probiotics and/or prebiotics ranges from intestinal infections (e.g., rotavirus infection) to extraintestinal infections (e.g., urinary infections) (cite) to allergic disorders (e.g., asthma); in other cases, these agents have been used prophylactically, e.g., to prevent colon cancer[66]. The strongest clinical data comes from trials of probiotics and prebiotics in the treatment of intestinal infections, inflammatory bowel disease, and irritable bowel syndrome [69]. Despite their widespread usage, knowledge of the putative mechanism of action of probiotics and prebiotics is limited. Most mechanistic studies in this area have centered upon production of antimicrobial substances to inhibit colonization by pathogens, enhance the mucosal barrier function, and downregulate mucosal inflammation [69]. It is particularly interesting that, despite the growing awareness of how gut microbes contribute to energy balance and despite the administration of probiotics/prebiotics as nutritional supplements, little research on this topic has focused on how these agents specifically impact nutrition, metabolism, or energy balance.

Several studies have been conducted to test the hypothesis that outcomes in critically ill patients can be improved by administering probiotics and prebiotics. These studies, including a randomized trial comparing the effects of early enteral nutrition with and without prebiotic supplementation, indicate that the incidence of sepsis and multi-organ dysfunction syndrome among patients with severe pancreatitis is lower after treatment with probiotics/prebiotics [70]. However, in 2008, the Dutch Acute Pancreatitis Study Group released results of a well-publicized multicenter, randomized, controlled study demonstrating increased mortality among patients with severe acute pancreatitis that received probiotic prophylaxis. The increased mortality was attributed to a high incidence of intestinal ischemia, although a direct link between the probiotic and bowel ischemia was not proven [71]. A subsequent meta-analysis concluded that probiotics do not influence mortality in the treatment of acute pancreatitis [72], however, the results of the Dutch study have raised important questions about the whether and how probiotics should be administered to vulnerable populations. Nonetheless, several other studies conducted in

surgical and medical ICUs, document improved outcomes after probiotic administration following trauma, liver transplant, and ICU admission for severe sepsis [73].

As noted, data regarding the safety and efficacy of probiotic and prebiotic administration are limited. Potential safety issues involved with manipulation of the microbiota with probiotics/prebiotics include probiotic-induced disease and antibiotic resistance [73]. Even if questions remain about efficacy and optimal route of delivery, it is generally accepted that probiotic administration in healthy individuals is safe. However, there is little understanding of how to approach these issues in the ICU. While probiotics have indeed been safely administered to vulnerable hospitalized populations such as neonates and transplant recipients, the significance of the results of the Dutch pancreatitis study cannot be overemphasized. They serve as a powerful reminder of the seemingly obvious fact that administering live microbial organisms to unhealthy patients might be dangerous, particularly when so little is known about the putative mechanism of action. The importance of exercising caution is further underscored by the scant federal regulation of commercial interests in this area.

Modulating the local gut microenvironment

Another possible approach to improve outcomes for critically ill patients is to manipulate the intestinal microenvironment to maintain the local microbial ecology of the GI tract indirectly. It is well established that the use of vasoactive pressors, antibiotics, and highly processed nutrients will change not only the local microbiota, but also pH, oxygen tension, SCFA production, and various critical micronutrients that maintain the health of normal intestinal microbes. Our group and others have shown that maintenance of a more acidic intestinal pH through the course of surgical injury and administration of oral pH solutions enhance local intestinal immunity and prevent lethal gut-derived sepsis [74]). Most recently we have shown that surgical injury causes a rapid depletion of mucus phosphate, thereby inducing certain strains of pathogenic bacteria to upregulate their virulence against the intestinal epithelial barrier [75]. Most bacteria that cause serious infections in ICU patients are equipped with exquisite sensory mechanisms to detect the level of local phosphate concentration. Phosphate concentration is a key trigger by which bacteria activate their virulence machinery to, in some cases, cause lethal sepsis. When phosphate levels are high at sites of local microbial colonization, such as the intestinal mucus, microbes use the PhoB phosphosensory/phosphoregulatory system to repress virulence activation. However, during phosphate depletion, the PhoB system is derepressed and virulence is activated even to the point of tissue invasion, immune activation, and organ failure [75]. The PhoB and analogous systems are highly conserved among microbes and offer an opportunity for clinicians to understand the precise host signals that trigger microbes to transform from indolent colonizers to lethal pathogens rapidly. We have shown in animal studies that maintenance of local phosphate concentration can suppress virulence activation among highly pathogenic bacteria such as *P. aeruginosa* even during periods of severe physiologic stress [74]. This also appears to be the case for other pathogens such as *C. albicans* and *E. faecalis* (unpublished observations). Therefore, providing therapies at the microenvironmental level could be a novel approach to create molecular diplomacy between pathogen and host through the course of severe physiologic stress such as that which occurs during human critical illness.

CONCLUSIONS

The intersection between the microbiome, nutrition, and critical illness will undoubtedly grow more interesting in the coming years. While the studies discussed in this paper provide clear evidence that gut microbes contribute to human nutrition and metabolism, it is too early to know if this information will be translated into meaningful improvements in current practice patterns. However, it is easy to identify clinical scenarios in critical care that are

likely to be impacted by this growing field of study; these topics include achieving positive nitrogen balance, managing hyperglycemia and cholestasis, and reducing the incidence of infectious complications during critical illness.

At present, a few concluding points can be safely made. First, it is apparent that future evaluations of human nutritional status during critical illness should include consideration of the gut microbiota. Second, it will be important to conduct the necessary studies to understand how the microbial ecology of the human body is altered during critical illness. Third, opportunities to manipulate the gut microbes in hospitalized patients are already presenting themselves, and the efficicacy of such interventions must be rigorously evaluated by multidisciplinary teams of clinicians and scientists with a solid understanding of microbial behavior.

Acknowledgments

This work was supported by Grant No. GM62344-11 (JCA) from the National Institutes of Health.

References

1. Martens J, Barg H, Warren MJ, et al. Microbial production of vitamin B12. Appl Microbiol Biotechnol. 2002; 58:275–285. [PubMed: 11935176]
2. Zoetendal EG, RajilicStojanovic M, de Vos WM. High-throughput diversity and functionality analysis of the gastrointestinal tract microbiota. Gut. 2008; 57(11):1605–1615. [PubMed: 18941009]
3. Pace N. The universal nature of biochemistry. Proc Natl Acad Sci USA. 2000; 98(3):805–8. [PubMed: 11158550]
4. Peterson J, Garges S, et al. NIH HMP Working Group. The NIH human microbiome project. Genome Res. 2009; 19(12):2317–2323. [PubMed: 19819907]
5. The Human Microbiome Jumpstart Reference Strains Consortium. A catalog of reference genomes from the human microbiome. Science. 2010; 328(5981):994–999. [PubMed: 20489017]
6. Qin J, Li R, Raes J, et al. A human gut microbial gene catalogue established by metagenomic sequencing. Nature. 2010; 464(7285):59–65. [PubMed: 20203603]
7. Ley RE, Peterson DA, Gordon JI. Ecological and evolutionary forces shaping microbial diversity in the human intestine. Cell. 2006; 124(4):837–848. [PubMed: 16497592]
8. Gill SR, Pop M, Deboy RT, et al. Metagenomic analysis of the human distal gut microbiome. Science. 2006; 312(5778):1355–1359. [PubMed: 16741115]
9. Hooper LV, Midtvedt T, Gordon JI. How host-microbial interactions shape the nutrient environment of the mammalian intestine. Annu Rev Nutr. 2002; 22:283–307. [PubMed: 12055347]
10. Xu J, Bjursell MK, Himrod J, et al. A genomic view of the human-bacteroides thetaiotaomicron symbiosis. Science. 2003; 299(5615):2074–2076. [PubMed: 12663928]
11. Manson JM, Rauch M, Gilmore MS. The commensal microbiology of the gastrointestinal tract. GI Microbiota and Regulation of the Immune System. 2008:15–28.
12. Turnbaugh PJ, Ley RE, Hamady M, et al. The human microbiome project. Nature. 2007; 449(7164):804–810. [PubMed: 17943116]
13. Wong JM, de Souza R, Kendall CW, et al. Colonic health: Fermentation and short chain fatty acids. J Clin Gastroenterol. 2006; 40(3):235–43. [PubMed: 16633129]
14. Macfarlane S, Macfarlane GT. Regulation of short-chain fatty acid production. Proc Nutr Soc. 2003; 62(1):67–72. [PubMed: 12740060]
15. Flint HJ, Bayer EA, Rincon MT, et al. Polysaccharide utilization by gut bacteria: Potential for new insights from genomic analysis. Nat Rev Microbiol. 2008; 6(2):121–31. [PubMed: 18180751]
16. Hooper LV, Xu J, Falk PG, et al. A molecular sensor that allows a gut commensal to control its nutrient foundation in a competitive ecosystem. Proc Natl Acad Sci U S A. 1999; 96(17):9833–9838. [PubMed: 10449780]

17. Raj T, Dileep U, Vaz M, et al. Intestinal microbial contribution to metabolic leucine input in adult men. J Nutr. 2008; 138(11):2217–21. [PubMed: 18936222]
18. Metges CC. Contribution of microbial amino acids to amino acid homeostasis of the host. J Nutr. 2000; 130(7):1857S–64S. [PubMed: 10867063]
19. Metges CC, Petzke KJ. Utilization of essential amino acids synthesized in the intestinal microbiota of monogastric mammals. Br J Nutr. 2005; 94(5):621–2. [PubMed: 16277760]
20. Torrallardona D, Harris CI, Fuller MF. Pigs' gastrointestinal microflora provide them with essential amino acids. J Nutr. 2003; 133(4):1127–31. [PubMed: 12672930]
21. Stewart GS, Smith CP. Urea nitrogen salvage mechanisms and their relevance to ruminants, non-ruminants and man. Nutr Res Rev. 2005; 18(1):49–62. [PubMed: 19079894]
22. Bergen WG, Wu G. Intestinal nitrogen recycling and utilization in health and disease. J Nutr. 2009; 139(5):821–5. [PubMed: 19282369]
23. Stewart GS, Smith CP. Urea nitrogen salvage mechanisms and their relevance to ruminants, non-ruminants and man. Nutr Res Rev. 2005; 18(1):49–62. [PubMed: 19079894]
24. Levenson SM, Crowley LV, Horowitz RE, et al. The metabolism of carbon-labeled urea in the germ free rat. J Biol Chem. 1959; 234(8):2061–2. [PubMed: 13673014]
25. Walser M, Bodenlos LJ. Urea metabolism in man. J Clin Invest. 1959; 38:1617–1626. [PubMed: 13842664]
26. Waterlow JC. The mysteries of nitrogen balance. Nutr Res Rev. 1999; 12(1):25–54. [PubMed: 19087445]
27. Steinbrecher HA, Griffiths DM, Jackson AA. Urea production in normal breast-fed infants measured with primed/intermittent oral doses of [15N, 15N]urea. Acta Paediatr. 1996; 85(6):656–662. [PubMed: 8816198]
28. Forrester T, Badaloo AV, Persaud C, et al. Urea production and salvage during pregnancy in normal jamaican women. Am J Clin Nutr. 1994; 60(3):341–346. [PubMed: 8074063]
29. Schwartz, SI.; Brunicardi, FC. Schwartz's Principles of Surgery. 9. New York: McGraw-Hill, Medical Pub. Division; 2010.
30. Backhed F, Ding H, Wang T, et al. The gut microbiota as an environmental factor that regulates fat storage. Proc Natl Acad Sci U S A. 2004; 101(44):15718–15723. [PubMed: 15505215]
31. Turnbaugh PJ, Backhed F, Fulton L, et al. Diet-induced obesity is linked to marked but reversible alterations in the mouse distal gut microbiome. Cell Host Microbe. 2008; 3(4):213–23. [PubMed: 18407065]
32. Velagapudi VR, Hezaveh R, Reigstad CS, et al. The gut microbiota modulates host energy and lipid metabolism in mice. J Lipid Res. 2010; 51(5):1101–12. [PubMed: 20040631]
33. Hill MJ. Intestinal flora and endogenous vitamin synthesis. Eur J Cancer Prev. 1997; 6 (Suppl 1):S43–5. [PubMed: 9167138]
34. Said HM, Mohammed ZM. Intestinal absorption of water-soluble vitamins: An update. Curr Opin Gastroenterol. 2006; 22(2):140–6. [PubMed: 16462170]
35. Tilg H, Moschen AR, Kaser A. Obesity and the microbiota. Gastroenterology. 2009; 136(5):1476–83. [PubMed: 19327360]
36. Ley RE, Backhed F, Turnbaugh P, et al. Obesity alters gut microbial ecology. Proc Natl Acad Sci U S A. 2005; 102(31):11070–11075. [PubMed: 16033867]
37. Turnbaugh PJ, Ley RE, Mahowald MA, et al. An obesity-associated gut microbiome with increased capacity for energy harvest. Nature. 2006; 444(7122):1027–1031. [PubMed: 17183312]
38. Turnbaugh PJ, Hamady M, Yatsunenko T, et al. A core gut microbiome in obese and lean twins. Nature. 2009; 457(7228):480–484. [PubMed: 19043404]
39. Ley RE, Turnbaugh PJ, Klein S, et al. Microbial ecology: Human gut microbes associated with obesity. Nature. 2006; 444(7122):1022–1023. [PubMed: 17183309]
40. Nadal I, Santacruz A, Marcos A, et al. Shifts in clostridia, bacteroides and immunoglobulin-coating fecal bacteria associated with weight loss in obese adolescents. Int J Obes. 2008; 33(7):758–767.
41. Zhang H, DiBaise JK, Zuccolo A, et al. Human gut microbiota in obesity and after gastric bypass. Proc Natl Acad Sci U S A. 2009; 106(7):2365–2370. [PubMed: 19164560]

42. Walker AW, Ince J, Duncan SH, et al. Dominant and diet-responsive groups of bacteria within the human colonic microbiota. The ISME Journal. 2010
43. Tennant B, Malm OJ, Horowitz RE, et al. Response of germfree, conventional, conventionalized and E. coli monocontaminated mice to starvation. J Nutr. 1968; 94(2):151–60. [PubMed: 4866341]
44. Tannock GW, Savage DC. Influences of dietary and environmental stress on microbial populations in the murine gastrointestinal tract. Infection and Immunity. 1974; 9(3):591–8. [PubMed: 4593471]
45. Bjornvad CR, Thymann T, Deutz NE, et al. Enteral feeding induces diet-dependent mucosal dysfunction, bacterial proliferation, and necrotizing enterocolitis in preterm pigs on parenteral nutrition. Am J Physiol Gastrointest Liver Physiol. 2008; 295(5):G1092–103. [PubMed: 18818317]
46. Deplancke B, Vidal O, Ganessunker D, et al. Selective growth of mucolytic bacteria including clostridium perfringens in a neonatal piglet model of total parenteral nutrition. Am J Clin Nutr. 2002; 76(5):1117–1125. [PubMed: 12399288]
47. Crawford PA, Crowley JR, Sambandam N, et al. Regulation of myocardial ketone body metabolism by the gut microbiota during nutrient deprivation. Proc Natl Acad Sci USA. 2009; 106(27):11276–81. [PubMed: 19549860]
48. Costello E, Gordon JI, Secor SM, et al. Postprandial remodeling of the gut microbiota in burmese pythons. The ISME Journal. 2010; 4(11):1375–85. [PubMed: 20520652]
49. Shimizu K, Ogura H, Goto M, et al. Altered gut flora and environment in patients with severe SIRS. J Trauma. 2006; 60(1):126–33. [PubMed: 16456446]
50. Heyland D, Mandell LA. Gastric colonization by gram-negative bacilli and nosocomial pneumonia in the intensive care unit patient. evidence for causation. Chest. 1992
51. Kerver AJ, Rommes JH, Mevissen-Verhage E, et al. Prevention of colonization and infection in critically ill patients: A prospective randomized study. Crit Care Med. 1988; 16(11):1087–93. [PubMed: 3168500]
52. Kerver A, Rommes JH, Mevissen-Verhage E. Colonization and infection in surgical intensive care patients:a prospective study. Intensive care Med. 1987
53. Ubeda C, Taur Y, Jenq RR, et al. Vancomycin-resistant enterococcus domination of intestinal microbiota is enabled by antibiotic treatment in mice and precedes bloodstream invasion in humans. J Clin Invest. 2010; 120(12):4332–4341. [PubMed: 21099116]
54. Marshall JC, Christou NV, Meakins JL. The gastrointestinal tract. the “undrained abscess” of multiple organ failure. Ann Surg. 1993; 218(2):111–119. [PubMed: 8342990]
55. Dethlefsen L, Huse S, Sogin ML, et al. The pervasive effects of an antibiotic on the human gut microbiota, as revealed by deep 16S rRNA sequencing. PLoS Biol. 2008; 6(11):e280. [PubMed: 19018661]
56. Croswell A, Amir E, Teggatz P, et al. Prolonged impact of antibiotics on intestinal microbial ecology and susceptibility to enteric salmonella infection. Infection and Immunity. 2009; 77(7): 2741–53. [PubMed: 19380465]
57. Antonopoulos DA, Huse SM, Morrison HG, et al. Reproducible community dynamics of the gastrointestinal microbiota following antibiotic perturbation. Infect Immun. 2009; 77(6):2367–2375. [PubMed: 19307217]
58. Lupp C, Robertson ML, Wickham ME, et al. Host-mediated inflammation disrupts the intestinal microbiota and promotes the overgrowth of enterobacteriaceae. Cell Host Microbe. 2007; 2(3): 204. [PubMed: 18030708]
59. Kohler JE, Zaborina O, Wu L, et al. Components of intestinal epithelial hypoxia activate the virulence circuitry of pseudomonas. Am J Physiol Gastrointest Liver Physiol. 2005; 288(5):G1048–54. [PubMed: 15550562]
60. Canani RB, Terrin G. Gastric acidity inhibitors and the risk of intestinal infections. Curr Opin Gastroenterol. 2010; 26(1):31–5. [PubMed: 19907324]
61. Alverdy J, Zaborina O, Wu L. The impact of stress and nutrition on bacterial-host interactions at the intestinal epithelial surface. Curr Opin Clin Nutr Metab Care. 2005; 8(2):205–9. [PubMed: 15716801]

62. Khoruts A, Sadowsky MJ. Therapeutic transplantation of the distal gut microbiota. Mucosal Immunol. 2011; 4(1):4–7. [PubMed: 21150894]
63. Oostdijk EA, de Smet A, Blok HE, et al. Ecological effects of selective decontamination on resistant gram-negative bacterial colonization. Am J Respir Crit Care Med. 2010; 181(5):452–7. [PubMed: 19965807]
64. de Smet A, Kluytmans JA, Cooper BS, et al. Decontamination of the digestive tract and oropharynx in ICU patients. N Engl J Med. 2009; 360(1):20–31. [PubMed: 19118302]
65. Stoutenbeek CP, van Saene H, Little RA, et al. The effect of selective decontamination of the digestive tract on mortality in multiple trauma patients: A multicenter randomized controlled trial. Intensive Care Med. 2007; 33(2):261–70. [PubMed: 17146635]
66. Santosa S, Farnworth E, Jones PJ. Probiotics and their potential health claims. Nutr Rev. 2006; 64(6):265–274. [PubMed: 16808112]
67. Douglas LC, Sanders ME. Probiotics and prebiotics in dietetics practice. J Am Diet Assoc. 2008; 108(3):510–21. [PubMed: 18313433]
68. Fooks LJ, Gibson GR. Probiotics as modulators of the gut flora. Br J Nutr. 2002; 88 (Suppl 1):S39–49. [PubMed: 12215180]
69. Quigley EM. Prebiotics and probiotics; modifying and mining the microbiota. Pharmacol Res. 2010; 61(3):213–8. [PubMed: 20080184]
70. Manzanares W, Hardy G. The role of prebiotics and synbiotics in critically ill patients. Curr Opin Clin Nutr Metab Care. 2008; 11(6):782–9. [PubMed: 18827584]
71. Besselink MG, van Santvoort HC, Buskens E, et al. Probiotic prophylaxis in predicted severe acute pancreatitis: A randomised, double-blind, placebo-controlled trial. Lancet. 2008; 371(9613):651–659. [PubMed: 18279948]
72. Sun S, Yang K, He X, et al. Probiotics in patients with severe acute pancreatitis: A meta-analysis. Arch Surg. 2009; 394(1):171–177.
73. Morrow LE. Probiotics in the intensive care unit. Curr Opin Crit Care. 2009; 15(2):144–8. [PubMed: 19300087]
74. Zaborina O, Zaborin A, Romanowski K, et al. Host stress and virulence expression in intestinal pathogens: development of therapeutic strategies using mice and C. elegans. Curr Pharm Des. 2011 Epub ahead of print April 6.
75. Long J, Zaborina O, Holbrook C, Zaborin A, Alverdy J. Depletion of intestinal phosphate after operative injury activates the virulence of P aeruginosa causing lethal gut-derived sepsis. Surgery. 2008; 144(2):189–97. [PubMed: 18656625]