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Review article

Probiotic and synbiotic therapy in the critically ill: State of the art

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ABSTRACT

Recent medical history has largely viewed our bacterial symbionts as pathogens to be eradicated rather than as essential partners in optimal health. However, one of the most exciting scientific advances in recent years has been the realization that commensal microorganisms (our microbiome) play vital roles in human physiology in nutrition, vitamin synthesis, drug metabolism, protection against infection, and recovery from illness. Recent data show that loss of "health-promoting" microbes and overgrowth of pathogenic bacteria (dysbiosis) in patients in the intensive care unit (ICU) appears to contribute to nosocomial infections, sepsis, and poor outcomes. Dysbiosis results from many factors, including ubiquitous antibiotic use and altered nutrition delivery in illness. Despite modern antibiotic therapy, infections and mortality from often multidrug-resistant organisms are increasing. This raises the question of whether restoration of a healthy microbiome via probiotics or synbiotics (probiotic and prebiotic combinations) to intervene on ubiquitous ICU dysbiosis would be an optimal intervention in critical illness to prevent infection and to improve recovery. This review will discuss recent innovative experimental data illuminating mechanistic pathways by which probiotics and synbiotics may provide clinical benefit. Furthermore, a review of recent clinical data demonstrating that probiotics and synbiotics can reduce complications in ICU and other populations will be undertaken. Overall, growing data for probiotic and symbiotic therapy reveal a need for definitive clinical trials of these therapies, as recently performed in healthy neonates. Future studies should target administration of probiotics and synbiotics with known mechanistic benefits to improve patient outcomes. Optimally, future probiotic and symbiotic studies will be conducted using microbiome signatures to characterize actual ICU dysbiosis and determine, and perhaps even personalize, ideal probiotic and symbiotic therapies.

The medical community has placed significant emphasis on the eradication of bacteria in humans for many years. However, we should now be considering how to preserve or re-establish the health-promoting bacteria that define a great deal of what makes us human ... and healthy. Undoubtedly, one of the most exciting scientific advances in recent years has been the realization that our bacterial symbionts or commensal microorganisms (our microbiome) play essential roles in our physiology as humans in a range of functions including nutritional state, vitamin synthesis, drug metabolism, protection against infection, and recovery from illness [1,2]. A surprising finding is that disruption of the homeostasis of the commensal bacteria, known as "dysbiosis," may be as vital as host genetics in the development of a range of diseases such as inflammatory bowel disease, obesity, diabetes, neurologic or mental illness, cardiovascular disease, and as recently shown, critical illness [2]. This suggests that we may be able to predict risk for disease, and prevent or even cure disease by regulating the bacteria that live on and within us. Very recent advances in microbiome-sequencing methods have resulted in an unprecedented growth in understanding this vital and dynamic part of what makes us human [3]. This new discovery raises the question of whether restoration of a healthy microbiome via probiotics or synbiotics (probiotic and prebiotic combinations) to intervene on the ubiquitous disruption of our healthy microbiome by the environment created by critical illness would be an optimal intervention in intensive care unit (ICU) patients to prevent subsequent infection and improve recovery.

Is our current approach to infection in the ICU and illness working?

A great deal of effort is spent in eradicating bacteria and other microbial, fungal, and viral species in the ICU. Data from the US Centers for Disease Control and Prevention show that 55% of all

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hospitalized patients receive an antibiotic during their stay, and in the ICU, this number rises to ~70%. This observation has been confirmed by recent multinational ICU survey data from >14 000 patients in >1200 ICUs, finding 51% of patients were considered to be infected on day of survey and a striking 71% were receiving antibiotic treatment [4]. The hospital mortality rate of patients with infections was found to be more than twice that of non-infected patients (33 versus 15%). Without doubt, infection and sepsis remain major drivers of morbidity and mortality, despite widespread ICU antimicrobial therapy use and advances in hospital care [4]. As recently described, it appears likely that antibiotic use has in some way contributed to the impressive 22-fold decrease in crude mortality rates for infectious diseases in the United States between 1900 and 1980 [5]. Yet, it is quite troubling that mortality rates from infectious disease up to 1996 increased by 50%, with the rate of septicemia nearly doubling [5]. It is unclear whether earlier reductions in mortality and longer life expectancy were primarily due to antibiotic innovations, or more likely, a result of improved public health and health education.

Recent evidence suggests that >35% of antibiotic regimens are unnecessary or not compliant with the latest guidelines [6]. This inappropriate and excessive antibiotic use is leading to the emergence of an increasing number of multidrug-resistant (MDR) bacterial infections. The incidence of MDR infections is rising rapidly both in the United States and worldwide [7]. A recent *New England Journal of Medicine* publication estimates that antibiotic-resistant MDR *Clostridium difficile* occurs in >450, 000 patients per year in the United States alone [7]. In addition, MDR infections are becoming increasingly lethal to hospitalized patients. *C. difficile* is estimated to contribute to ~30 000 deaths annually in the United States [7,8]. And again, this is emphasized by recent data showing mortality rates from infectious disease increasing by 50%, and the septicemia rate nearly doubling [5]. Thus, more advanced antibiotics do not appear to be consistently translating to continued improvements in survival from infectious disease in the modern health care environment, but rather to often increasingly aggressive MDR organisms and emergence of increasingly lethal pathogens like *C. difficile*. Is it time to rethink our strategy regarding bacteria and microbiota in our ICU patients?

The evolving role for probiotics and synbiotics to promote recovery in the ICU

Is it possible that to improve critical care outcomes we should be giving "friendly" bacteria to ICU patients, rather than eradicating them? Perhaps our success as a species is based on the evolved presence of the trillions of bacterial symbionts that each of us live in harmony with each day? This raises the key question of whether we should be shifting our focus to treating dysbiosis by restoring a normal, healthy microbiome in ICU patients in order to fight infection and improve ICU recovery. As has been described, this may be essential because it is, in many ways, our bacteria that make us "human," as there are far more microbial genes in our bodies then human genes and more bacterial cells then human cells in each of us right now [9].

As suggested by Loupazone et al. [10], perhaps we need to use probiotics and synbiotics to "resod" the healthy "microbial lawn" that is decimated by critical illness (and antibiotics). This concept is supported at a mechanistic level, as described here. The gut has been hypothesized to be the "motor" driving systemic inflammation and organ failure in the ICU [11]. To modulate this "motor" of systemic inflammation, it has been hypothesized that repletion of health-promoting bacteria via probiotics and synbiotics may be a promising intervention to maintain gut integrity and to prevent pathologic alterations in the gut (and other body sites) microbiota or "dysbiosis," which are the fuel for this "motor" of ongoing injury [12–14].

Mechanistic role of probiotics and synbiotics in critical care

Mice that receive fecal transplants (microbial communities) from obese or malnourished humans phenocopy the disease associated with the transplant, indicating that abnormal microbial communities drive nutritionally related diseases [15,16]. Similarly, the microbiota are linked to immune-mediated inflammatory diseases like rheumatoid arthritis and multiple sclerosis and inflammatory bowel diseases (IBD) like ulcerative colitis and Crohn's disease [17]. Although the microbiota can induce inflammatory responses, certain members of the human intestinal microbiota generate anti-inflammatory proteins and molecules that can attenuate local or systemic inflammation. For example, *Faecalibacterium prausnitzii* produces a peptide that can attenuate dinitrobenzene sulfonic acid-induced colitis in mice [18]; however, it remains unknown if this same bacterium attenuates systemic inflammation during sepsis. Similarly, human macrophage and neutrophil cell culture models have shown that short-chain fatty acids (SCFAs), which are predominantly made by the anaerobic microbiota residing in the colon, act as anti-inflammatory mediators by regulating cytokine release and prostaglandin production [19]. Based on these studies, we speculate that loss of these beneficial microbial communities may impair human nutrition and activate abnormal inflammatory responses. Therefore, correcting or supplementing microbiota communities may restore nutritional deficiencies and anti-inflammatory factors and promote human health.

Abnormal microbiome assemblies and loss of beneficial microbes (dysbiosis) that support healthy nutrient metabolism and absorption are associated with critical illness. Indeed, as discussed further here, our group has shown that critical illness and ICU admission are associated with significant and rapid dysbiosis of the microbiome [20]. This dysbiosis in critical illness was most evident by the marked loss of the beneficial bacterium, *F. prausnitzii.* This bacterium has become of intense interest to the IBD field because its loss is strongly associated with the onset or relapse of IBD [21,22] and for its capacity to generate an anti-inflammatory peptide that suppresses local inflammation and promotes epithelial barrier function in part through the production of SCFA [18,23–25]. However, unlike IBD, it remains unknown if loss of *F. prausnitzii* or other beneficial microorganisms contribute to critical illness. There is currently intense interest in using probiotic therapies to support nutrition and metabolic health in both healthy and critically ill patients [26,27]. Previous work has demonstrated that daily oral gavage of the probiotic bacteria *Lactobacillus rhamnosus* GG (LGG) and *Bifidobacterium longum* promote survival in septic mice [28]. However, the mechanisms of these probiotics that confer protection from sepsis remain unknown. Oral supplementation of probiotics represents a logical approach to critical illness therapy given the significant contributions the microbiota make in nutrient production, absorption, and modulation of inflammation. Yet, a significant obstacle that stalls use of probiotic therapies in critical care is the lack of animal models that have identified specific molecular mechanisms that mediate probiotic contributions to human health, especially in states of acute illness. It is important to note that beneficial mechanisms of probiotics are specific to particular species and possibly even subspecies or strain; these therapeutic properties cannot be assumed for other species within the same genus. Furthermore, some probiotics may prove beneficial in some physiological states but detrimental in others (e.g., *Saccharomyces boulardii*). Therefore, each probiotic species, subspecies, or strain must

be analyzed in the context of critical illness to determine the beneficial mechanisms. By understanding these hypothesized mechanisms as described in Figure 1 and outlined here, we may be able to derive new nutritional probiotic and synbiotic therapies that can support patient health and improve ICU outcome. In the following, we discuss host responses to the microbiota and known mechanisms of probiotic action that may benefit critically ill patients and improve ICU outcome.

Mechanisms of probiotic protection from infection and sepsis

Maintaining epithelial barrier function

The intestinal epithelium is comprised of a single layer of columnar epithelial cells that detect and respond to dietary nutrients and molecular cues within the environment. Enterocytes comprise the largest cell population in the small intestinal epithelia and these harvest dietary nutrients from the lumen to maintain energy homeostasis for all animals. Secretory cells, like goblet and Paneth cells, secrete proteins into the luminal environment to protect the epithelia from the harmful microbes within the lumen [29]. For instance, goblet cells produce a highly glycosylated protein called *MUC2*, which serves as a large net and attenuates Brownian motion of molecules like microbe-associated molecular patterns and blocks the invasion of the majority of the microbiome [30]. Probiotics like *Lactobascilli acidophilus* and *LGG* have been shown to induce *MUC2* expression in human intestinal cell lines [31,32]; however, in vivo experiments with a 14-d supplementation of the probiotic cocktail VSL#3 (which contains a *Lactobacilli*) in mice have shown no effect on *MUC2* expression or mucus layer thickness [33]. Conversely, a 7-d supplementation with VSL#3 in rats resulted in 60-fold increase in *MUC2* expression with an increase in mucus layer thickness [34]. In critical illness, experimental models of pneumonia have shown that *LGG* improves intestinal permeability in part by inducing *MUC2* gene expression and protein production [27] (Fig. 1A).

In the small intestine, Paneth cells serve as the primary caretaker and protector of the intestinal stems cells located in the crypts of the small intestine [1,35]. Paneth cells sense and respond to fluctuations within the microbiota [36]. Upon infection of pathogenic bacteria, Paneth cells become filled with granulae that contain lysozyme and antimicrobial peptides [37]. These antimicrobial peptides include α-defensins and the C-type lectins, like RegIIIg, which serve as part of the innate immune system response. These molecules disrupt microbial membrane integrity and kill invading bacteria [37]. Previous work has shown that the probiotic, *Bifidobacterium breve* induces expression of *REGIII* genes and activates the defense mechanisms of Paneth cells [38]. Interestingly, overstimulation of Paneth cells and their overexpression of antimicrobial peptides and proinflammatory cytokines like IL17 A may result in intestinal barrier dysfunction and systemic inflammation [39,40], which could result in sepsis and multiple organ failure. Interestingly, *B. bifidum* attenuates the expression of antimicrobial peptides in rat models of necrotizing enterocolitis, indicating this probiotic may augment Paneth cell function and thereby promote intestinal barrier function [41] (Fig. 1A).

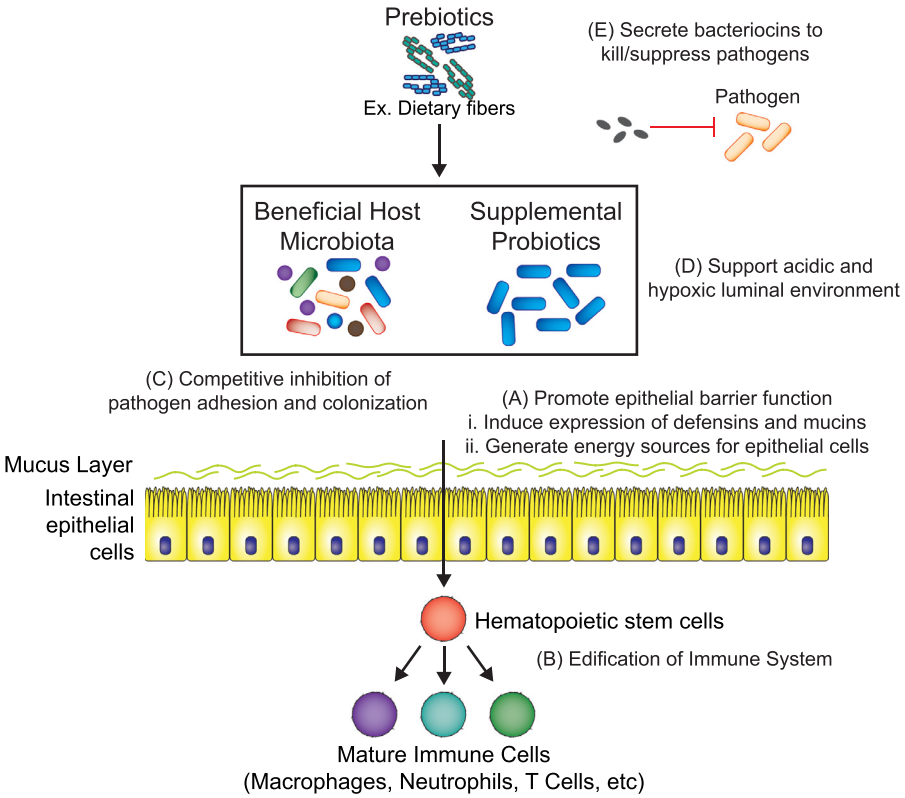

Fig. 1. Proposed mechanistic pathways for benefit of probiotics and probiotic/prebiotic combinations (synbiotics) in citical illness. The intestinal epithelia, the host immune system, and the microbiota maintain intestinal homeostasis and prevent pathogen infiltration or deter opportunistic microorganisms from becoming virulent. Critical illness is associated with fluctuation in the microbiota that result in a dysbiosis, which likely contributes to illness progression. Probiotic, prebiotic, and the combination of the two (termed *synbiotics*) treatment may help restore homeostasis and thereby prevent or treat critical illness. Beneficial host microbiota and probiotics have been shown to promote intestinal epithelial barrier function (A); edify the immune system (B); and out-compete pathogens for niches in the intestine by preventing pathogen adhesion and colonization (C), promoting an acidic and hypoxic environment that is unsuitable for pathogen colonization (D), secreting bacteriocins that kill or suppress pathogens. Prebiotics serve as energy sources for beneficial microbes and probiotics and help establish niches for these microorganisms.

Loss of intestinal epithelial barrier function has been associated with systemic inflammatory response syndrome, sepsis, and multiple organ failure [42,43]. Compromise of epithelial barrier function may result if the rate of epithelial cell apoptosis is greater than the rate of epithelial renewal (cellular proliferation and differentiation from crypt stem cells). Interestingly, oral gavage of the probiotic *LGG* or *B. longum* reduces sepsis-induced colonic epithelial cell apoptosis and promotes intestinal epithelial cell proliferation [28]. Furthermore, bacteremia is significantly reduced in septic mice that receive an oral gavage of *LGG* and *B. longum*. Together, these results suggest that probiotic supplementation promotes epithelial barrier function in septic mice in part by promoting epithelial renewal. Our best understanding of how the microbiota modulate epithelial renewal processes comes from the experiments performed in zebrafish and drosophila model organisms. In zebrafish, microbial communities secrete a factor that promotes proliferation through activation of the β-catenin signaling [44]. Interestingly, in *Drosophila melanogaster*, the microbiota and infectious bacteria induce expression of the *DUOX* gene, which generates reactive oxygen species as a part of the innate immune response; this oxidative burst in response to the microbiota induces epithelial renewal [45]. These results indicate that the innate immune response mediates microbial induction of the proliferation of intestinal epithelial cells. Similarly, antibiotic treatment results in cell-cycle arrest in colon epithelial cells, suggesting that the loss of beneficial microbial communities impairs epithelial proliferation [46].

Generating nutritional support for the host epithelial cells

Along the length of the intestine, both host and microbiota catabolize and solubilize complex macromolecules to usable energy sources such as peptides and fatty acids. The relative concentrations of these dietary molecules change along the length of the intestine and these correlate with epithelial and microbiota function. For example, the most proximal segments of the mammalian intestine function as the primary location for fatty acid and simple carbohydrate absorption [47]; similarly, the bacteria within these segments thrive by metabolizing these dietary molecules and contribute to epithelial absorption of these molecules [48–50]. Host-derived molecules like bile acids similarly contribute to nutrient absorption: Bile acids facilitate fatty acid digestion by breaking down large dietary fat droplets into micelles that are digestible by secreted lipases. The microbiota modify primary bile acids, adding different chemical groups to the structure of these molecules to generate secondary bile acids that are known to have varying effects on host metabolism [51,52]. Water and electrolytes are absorbed in the most distal part of the intestine [53], the colon, which also harbors the largest concentration of anaerobic bacteria. These anaerobic bacteria support colon health by catabolizing dietary fibers into SCFA, the primary energy source for colonocytes [54,55]. Similarly, bacterial generation of SCFAs drives β-oxidation in colonocytes, which supports epithelial barrier function; this enhanced β-oxidation promotes a hypoxic environment within the colon and prevents the establishment of pathogenic microbial communities [56] (Fig. 1D).

Changing the metabolic transcriptional landscape

It is well established that the intestinal microbiota influence gene transcription in several tissues and the chromatin landscape of the small intestine [57]. However, the transcriptional response during sepsis has not been sufficiently investigated. We understand that sepsis induces a burst of prolonged proinflammatory cytokine gene expression and protein production; only recently have we started to understand how sepsis effects *metabolic* gene expression. We predict metabolic gene transcription networks become suppressed in sepsis because a common theme in inflammation studies is that the activation of immune genes coincides with a suppression of metabolic gene programs [58]. Indeed, transcriptome profiles from septic primates demonstrate severe mitochondrial dysfunction and reduced β-oxidation in inflamed lung compared with healthy primates [59]. Therefore, simply providing energy (kcals), protein, and micronutrients to septic patients may not be enough to address their metabolic deficiencies as the metabolic transcription programs are likely suppressed in sepsis. It remains unknown if probiotic therapies in sepsis repair these dysfunctional metabolic transcription programs. However, some studies have indicated that probiotics and peacekeeping taxa that become depleted in patients in critical care, like *F. prausnitzii*, can augment and induce metabolic transcriptional programs. Indeed, a recent study has shown that *F. prausnitzii* supplementation induces fatty acid oxidation in the liver of mice fed high-fat diets [60]. Furthermore, the probiotic strain *L. johnsonii* similarly has been shown to promote metabolic gene expression and improve lipid metabolism [61]. These results indicate that probiotics may help improve nutrient harvest and repair the metabolic dysfunction commonly seen in the ICU.

Edification of the immune system

In addition to its effect on the innate immune response by the intestinal epithelia, as discussed earlier, the microbiota also modulate hematopoiesis and immune cell maturation and activation. For instance, in mice and zebrafish, the microbiota promote differentiation of innate immune cells: macrophages and neutrophils [62,63]. Expansion of these innate immunity cell types confers resistance to some infections [63]. Interestingly, the probiotic *L. rhamnosus* CRL1505 improves emergency neutrophil differentiation in immunocompromised malnourished mice [64]. This finding indicates probiotic supplementation may help protect at-risk and malnourished patients from developing sepsis. The microbiota also promotes neutrophil migration, which protects the host from injury and infection [62]. In animal models of critical care, neutrophil infiltration in the lungs is significantly higher in septic mice than in non-septic mice. Excitingly, oral gavage of probiotics attenuates this inflammation, leading to reduced neutrophil infiltration in the lung [65]. However, the exact mechanisms of how these probiotics reduce neutrophil infiltration and attenuate lung inflammation remain unknown. Perhaps they simply promote intestinal barrier function by the mechanisms discussed here, thereby limiting infectious bacteria from entering the blood and causing sepsis. Or, perhaps these probiotics communicate directly with the innate and adaptive immune systems to reduce systemic inflammation and promote extraintestinal organ function. More studies must be conducted to determine exact mechanisms of how enteric bacteria protect extraintestinal organs and attenuate systemic inflammation (Fig. 1B).

Experiments with germ-free mice (mice raised in the absence of a microbiota) have shown that commensal microbiota and probiotics edify the adaptive immune system. For example, *Bacteroides fragilis* mediates a healthy adaptive immunologic function by establishing proper T helper (Th)1/Th2 balance [66]. Furthermore, segmented filamentous bacterium promotes establishment of Th17 cell populations and confers resistance to the intestinal pathogen *Citrobacter rodentium* in mice [67]. Some studies have found that probiotics edify adaptive immunity in humans and its effects can be seen weeks to years after treatment ends. For instance, early exposure (neonate and infant exposure) to

probiotics has been shown to prevent immunoglobulin E-associated allergies in children 5 y of age who were delivered via cesarean [68]. Furthermore, one study found that probiotic *L. casei* results in increased T-cell activation 6 wk after discontinuation of probiotic ingestion in healthy adults [69]. Finally, studies in mice have shown that two doses of heat-sacrificed *L. casei* confers protection against immediate and future H1 N1, H3 N2, and rgH5 N1 viral infections [70]. Less is known about how the microbiota control the adaptive immune response in critical care. However, in a murine model of pneumonia, *LGG* supplementation has been shown to increase T-cell number in the lungs of *Pseudomonas aeruginosa*-infected mice [71] (Fig. 1B).

Other probiotic roles to prevent dysbiosis in intestinal lumen

Commensal microbes and probiotics not only tune host defenses to maintain intestinal homeostasis and resist invasion of pathogens, but they also directly change the luminal environment to inhibit pathogen colonization. For example, anaerobic fermentation in the colon offers major benefits for the host:

1. SCFA fermentation directly benefits the host by providing dietary nutrients for colonocytes (discussed previously).
2. SCFA fermentation also indirectly benefits the host by boosting β-oxidation in the colonyctes, which promotes hypoxic environments and inhibits colonization of harmful bacteria like *Enterobacteriaceae*.
3. Fermentation acids, like lactate, directly benefit the host by reducing luminal pH in the colon, which can inhibit pathagenic bacteria colonization [72] (Fig. 1D).

For example, bacterial culture assays have demonstrated that commercially available probiotics and some of their secreted metabolites inhibit *C. difficile* and clinical strains of *C. perfringens* growth [73]. Intestinal cell culture assays have demonstrated that members of the probiotic *Bifidobacterium* spp. synthesize organic acids that reduce media pH and inhibit *C. difficile* adhesion to epithelial cells [74,75] (Fig. 1C, D). In vitro experiments also have demonstrated that human infant isolates of *B. bifidum* generate a zone of inhibition that impedes pathogenic *Escherichia coli* strain O157:H7 growth and impairs pathogen adhesion to cultured intestinal epithelia [76]. Furthermore, bacterial culture studies indicate that mixtures of several probiotics function synergistically to inhibit pathogen growth [77]. Unfortunately, many of these studies failed to accurately recapitulate the complex ecology that persists in the mammalian intestine and which can only be accurately studied in model organisms. Excitingly, a novel in vivo study demonstrated that *L. salivarius* produces a bacteriocin that protects the host from *Listeria monocytogenes* [78] (Fig. 1E). These studies indicate that commensal microbes and probiotics may provide a direct benefit for the host by impairing pathogen establishment in the intestinal tract. We do not know if these mechanisms play a role in protecting and recovering from sepsis in humans; however, given that the intestine is thought to be the major source of septic infection [79,80], reseeding the intestine with probiotics that suppress pathogen growth represents a logical treatment for the microbial dysbiosis that occurs in critical care patients. These treatments may not only recover homeostasis within the gut, but as observed in animal models of critical care, they also may aid in the recovery from the systemic infection by attenuating inflammation and promoting organ function. Animal studies of critical care represent a cornerstone to better understand the mechanisms of probiotic protection in sepsis and will lead to novel hypotheses to test in clinical trials.

Review of current data for probiotic/synbiotic use in critical illness

Probiotic use in critical care has been the subject of an increasing number of clinical trials and meta-analysis focused on a range of outcomes after critical illness. Many clinical trials and meta-analysis efforts have focused on the role of probiotics in reducing ventilator-associated pneumonia (VAP). Despite promising data for probiotic use in reducing overall infections, the role of probiotics as a strategy to prevent VAP has been controversial. Recently, a Cochrane review of probiotic therapy specifically for VAP [81] found with a low-quality of evidence that probiotic therapy is associated with a reduction in VAP. In 2015, an updated meta-analysis from the Canadian Clinical Practice Guidelines Committee became available on www.criticalcarenutriton.com. This analysis evaluated 28 studies of probiotic therapy in critical care published up to late 2014. The new results indicate that probiotics continue to show a significant reduction in infections after critical illness (risk ratio [RR], 0.82; 95% confidence interval [CI], 0.69–0.97; $P = 0.02$, heterogeneity $I^2 = 41\%$) and a trend to reduction in VAP (RR, 0.74; 95% CI, 0.55–1.01; $P = 0.06$; heterogeneity $I^2 = 45\%$). There was a trend toward reduced ICU length of stay (LOS) when results of 14 trials were pooled (weighted mean difference, −3.26; 95% CI, −7.82 to 1.31; $P = 0.16$; heterogeneity $I^2 = 93\%$), although significant statistical heterogeneity existed in these data. No significant effect on mortality, hospital LOS, or other outcomes was noted. The reduction in infections disappeared when only high-quality studies were considered. Based on this analysis, the Canadian Clinical Practice Guidelines Committee concluded that the use of probiotics should be considered in critically ill patients. As a wide range of probiotic species and doses were used in these trials, no recommendation could be made for the dose or a particular type of probiotic, with the exception of *S. boulardii*, which should not be used as it has been considered to be unsafe in ICU patients [82]. Clearly, further large and well-designed clinical trials are needed to strengthen the recommendation of probiotic use and to confirm benefits in ICU patients.

Recently, we conducted the largest and most updated systematic review and meta-analysis evaluating the overall effects of probiotics and synbiotics in the critically ill [26]. We aggregated 30 randomized controlled trials enrolling 2972 critically ill adult patients receiving probiotics alone or synbiotics compared with placebo. When the results of the 14 trials reporting overall infections were pooled, probiotics were associated with a significant reduction in ICU-acquired infections (RR, 0.80; 95 % CI, 0.68–0.95; $P = 0.009$; heterogeneity $I^2 = 36\%$; $P = 0.09$). After aggregating data of nine trials reporting on VAP, probiotics showed an association with a significant reduction in the incidence of the disease (RR, 0.74; 95 % CI, 0.61–0.90; $P = 0.002$; $I^2 = 19\%$; $P = 0.27$) [26]. Furthermore, a significant reduction in the duration of antibiotic therapy, estimated to be ∼1 d, was found when data from four trials were pooled that reported on this outcome ($P = 0.0003$). Thus, clear benefits on the incidence of ICU-acquired infections and particularly on new episodes of VAP was shown. Our analysis did not show an effect of probiotic therapy on other relevant outcomes in critically ill patients such as mortality or hospital and ICU LOS [26]. A subgroup analyses demonstrated that probiotics appeared to be more effective in reducing nosocomial infections in patients at high risk for death, in those who received probiotics alone, and in studies treating with *L. plantarum* either alone or in a multispecies mixture [26].

Focusing on antibiotic-associated diarrhea, a recent meta-analysis in *JAMA* showed in 63 studies and >11 800 patients that probiotics reduced antibiotic-associated diarrhea by 40% [83]. A

recent Cochrane meta-analysis of probiotic use in *C. difficile* colitis and diarrhea demonstrated probiotics reduced *C. difficile*–associated diarrhea by 64% in patients taking antibiotics (23 studies, N = 4213) [84]. Probiotics also reduced risk for side effects known to be associated with antibiotic use in this analysis. In another key meta-analysis, probiotics were effective in primary prevention of *C. difficile*–associated diarrhea, but may not have clear efficacy in secondary prevention of *C. difficile*–associated diarrhea [85]. Finally, a small two-patient study demonstrated that supplementation with a multispecies bacterial mixture alleviated antibiotic-resistant *C. difficile* colitis within 2 to 3 d and persisted for ≥6 mo [86]. This 33 multispecies bacterial mixture (termed *MET-1*) also protects mice from *Salmonella* infection and promotes intestinal barrier function [87].

Finally, a landmark trial in the benefit of probiotic and symbiotic therapy against sepsis in infants was recently published in *Nature* [88]. Sepsis in infants leads to ~1 million deaths worldwide, with many occurring (but not unique) in developing countries [88]. This was a randomized double-blind, placebo-controlled trial of an oral synbiotic preparation (*L. plantarum* plus fructooligosaccharide) in 4556 rural Indian newborns. Results demonstrated a significant reduction in the primary outcome (combination of sepsis and death) in the treatment arm (RR, 0.60; 95% CI, 0.48–0.74). Significant reductions also were observed for culture-positive and culture-negative sepsis and lower respiratory tract infections. These findings suggest that a large proportion of neonatal sepsis in developing countries, and perhaps in other settings, could be effectively prevented using a synbiotic containing *L. plantarum* ATCC-202195. This is a defining trial for the symbiotic and probiotic field and provides a model for future large probiotic and symbiotic trials to emulate.

Concluding remarks: defining the ICU microbiome (dysbiosis) to target probiotic therapy

Characterization of ICU microbiome changes may provide key insight to guide development of diagnostic and therapeutic interventions with probiotics and synbiotics using microbiome signatures. Our research group recently completed a multicenter trial to characterize the effect critical illness on the microbiome [20]. The ICU Microbiome Trial assessed fecal and oral samples of 115 adult medical and surgical ICU patients, processed according to standardized Earth Microbiome Project protocols. We found critical illness was associated with an early and marked depletion of commensal microorganisms (dysbiosis) and, at the same time, a significant increase in a pathogen-dominant microbiome. More specifically, our results show when compared with healthy American Gut Subjects, critical illness is associated with rapid and distinct changes from a "healthy" fecal and oral microbiome [20]. Fecal ICU samples tended to have lower relative abundance of Firmicutes and increased relative abundance of Proteobacteria [20]. Large depletions were observed in organisms shown to confer anti-inflammatory benefits such as *Faecalibacterium,* which, as discussed previously, specifically is known to produce SCFAs that are vital to the gut. Conversely, many of the taxa, which increased, contain well-recognized pathogens (i.e., *Enterobacter* and *Staphylococcus).* This multicenter study confirms dysbiosis occurs in critically ill patients.

However, the cause of this dysbiosis remains unknown. We speculate that undue stress on the patient caused by antibiotic use, hospital interventions, and the course of illness (including malnutrition and oxidative stress) combined with the patient's genetic background contribute to this dysbiotic microbiome. This speculation is consistent with other critical care researchers who have suggested stress promotes bacterial mutagenesis and pathogenesis, enables opportunistic microbes to become virulent, induces inflammation, and breaches the intestinal epithelial barrier [89–92]. Importantly, the factors that contribute to dysbiosis also may determine how a patient responds to probiotic therapy. Furthermore, we and others speculate that a single probiotic isolate (or a combination of several probiotic isolates) could not restore symbiosis on its own. Instead, a combination of diet and the cooperative interactions and modifying activities between probiotics and the existing microbiota promote symbiosis [93]. Using these data, future probiotic therapies can be "targeted" to restore "health-promoting" microbes in ICU patients. We believe these are the first steps to "resodding" the lawn of the gut (and perhaps oropharynx) with beneficial bacteria lost after critical illness to improve outcomes. Given the growing body of mechanistic understanding for probiotic benefits, improved outcomes with synbiotics in neonatal sepsis observed in a recent large randomized trial in *Nature* and encouraging signals on clinical outcome from recent meta-analysis data, further studies are urgently needed to identify the ideal probiotic(s) or synbiotic(s) needed to improve ICU infectious and clinical outcomes. In particular, well-designed and powered large-scale multicenter clinical trials of probiotic and synbiotic therapy are needed.

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