

## Mini Review Article

## Critical illness and the role of the microbiome

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The number of microbes living within the intestinal lumen is similar to the number of all cells of human origin in the host. Although historically little attention has been paid to the massive microbial community residing inside each of us, the last few years have witnessed an explosion of information related to the role of the microbiome in the maintenance of health and in the pathogenesis of disease. Here, we review data suggesting that the microbiome is converted into a pathobiome in critical illness and potential strategies for targeting the microbiome for therapeutic gain in the intensive care unit.

**Key words:** Critical care, gut, intensive care unit, intestine, microbiome

## INTRODUCTION

Approximately 40 trillion microorganisms reside inside the intestine.<sup>1</sup> Under basal conditions, the microbiome is not simply an innocent bystander, living peacefully side-by-side with its human host. Rather, commensal microbes are health-promoting and play numerous diverse roles in the maintenance of human wellness. In contrast, the microbiome is severely altered in multiple disease states with the conversion of the health-inducing microbiome into a disease-promoting microbiome (also known as a pathobiome).<sup>2</sup> These perturbations are particularly pronounced in the intensive care unit (ICU), where the gut has long been hypothesized to be “the motor” of critical illness.<sup>3,4</sup>

The largest study to date on the microbiome in critical illness compared fecal samples from 115 adult ICU patients in four medical centers within 48 h of admission and at discharge or on ICU day 10 to 1,242 healthy patients.<sup>5</sup> Critically ill patients had rapid depletion of health-promoting organisms and overgrowth of known pathogens. Specifically, when examining phylum-level taxonomy, the common

gram-positive Firmicutes and Gram-negative Bacteroidetes were both decreased, as was *Faecalibacterium*, an anti-inflammatory organism. In contrast, potential pathogens such as *Enterobacter* and *Staphylococcus* were increased, and there was a relative increase in Proteobacteria. Phylogenetic diversity was also significantly decreased at discharge compared to admission. Of note, this study used 16S rRNA gene amplicon sequencing, which does not provide sufficient genomic resolution to identify bacterial species, but identifies genera instead. This is important because genera cannot distinguish between pathogenic and non-pathogenic species (*Staphylococcus aureus* versus other non-pathogenic *Staphylococci*, for instance).

Similarly, a prospective observational study of 34 ICU patients (both septic and non-septic) and 15 healthy controls showed a marked shift in fecal bacterial composition in critically ill patients with disappearance of bacteria genera with key functions in host metabolism.<sup>6</sup> Notably, there were extreme individual differences in 13 critically ill patients with a single bacterial genus making up more than 50% of the gut microbiota. However, no association was identified between microbial diversity, Firmicutes/Bacteroidetes ratio or Gram-positive/Gram-negative ratio and survival or complications. This remarkable loss of diversity mimics a study of 14 ICU patients that reported the emergence of ultra-low-diversity communities in 35% of patients containing only one to four bacterial taxa.<sup>7</sup> Similar to the above studies, at the phylum level, the communities most commonly contained *Enterococcus*, *Staphylococcus*, and *Enterobacter*. Notably, cultured stool samples correlated to the 16S rRNA

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analysis, but also revealed the emergence of *Candida albicans* and *Candida glabrata* in 75% of patients.

It is crucial to note that microbiome composition is not static but rapidly evolves over time in the ICU and with severity of illness. It was initially reported nearly 50 years ago that the prevalence of Gram-negative oropharyngeal bacteria is low in physiologically normal subjects despite hospital exposure but increases markedly in sick patients, and this increased prevalence correlates most closely with severity of illness.<sup>8</sup> More recently, a pilot study of 12 adult ICU patients examined stool samples on mechanically ventilated patients on days 1–2, 2–4, 5–8, and 7–10.<sup>9</sup> Bacteria belonging to the phyla Firmicutes and Bacteroidetes were predominant in all samples, but the percentages changed markedly over time. Notably, a Bacteroidetes/Firmicutes ratio of >10 was seen in four of six non-survivors, whereas a ratio of <0.1 was seen in one non-survivor. No survivor had a ratio of >10 or <0.1, although this small study was underpowered to draw any conclusions related to the relationship between this ratio and mortality.

The microbiome is also altered in critically ill children. A comparison of 37 pediatric ICU patients with a mean age of 2.9 years to both pediatric and adult reference datasets demonstrated that pediatric ICU patients had decreased diversity with enrichment at the genus level of *Enterococcus* and *Staphylococcus* at multiple body sites with depletion of commensals such as *Faecalibacterium* and *Ruminococcus* from the gut.<sup>10</sup> Notably, both alpha and beta diversity were unstable over time in patients followed longitudinally.

The microbiome is not restricted to the gut and multiple body sites contain microbes that have been implicated in critical illness. For instance, a study comparing 15 patients requiring mechanical ventilation to healthy subjects who had lower respiratory tract sampling by bronchoscopy showed that both upper and lower respiratory tract microbiota diversity were decreased within 24 h of intubation and further decreased over time.<sup>11</sup> Furthermore, in a study of patients admitted to the ICU after severe blunt trauma, smoking prior to the ICU admission was significantly associated with microbial composition both at ICU admission and at 48 h, and this also was associated with development of acute respiratory distress syndrome.<sup>12</sup> The lung microbiota was also reported to be altered in a mouse study of acute lung injury induced by lipopolysaccharide.<sup>13</sup>

In addition, emerging evidence suggests that gut-derived bacteria travel to other body sites in critical illness. Using culture-independent evidence in a murine model of sepsis, lung communities were dominated by gut-associated bacteria, and ecological analysis revealed the lower gastrointestinal tract as the likely source of post-sepsis lung bacteria (rather than the upper respiratory tract). In addition, gut-

specific bacteria were abundant in patients with acute respiratory distress syndrome.<sup>14</sup> Similarly, gut-associated bacteria were enriched in the brains of mice 5 days after abdominal sepsis, and this was associated with severity of neuroinflammation.<sup>15</sup>

The etiology behind the changes to the microbiome in critical illness is almost assuredly multifactorial. Plausible causes include changes induced by the critically ill state as well as unintentional side-effects of treatments of critical illness (e.g. antimicrobial therapy, opiates, proton pump inhibitors, and tube feeding). It is often difficult to uncouple the effects of critical illness from the impact of antibiotics on the microbiome in the ICU, as the majority of ICU patients receive antimicrobial therapy at some point during their hospitalization. Furthermore, as the microbiome acts as a major modulator of innate immunity, it is theoretically possible that antibiotics alter immune responsiveness by altering the microbiome (distinct from their intended antimicrobial action). In an attempt to model this, a proof-of-principle trial randomized 16 healthy men to receive either broad spectrum antibiotics or no treatment for 7 days followed by a single dose of endotoxin, designed to mimic a transient septic-like state.<sup>16</sup> As expected, microbial diversity was significantly diminished by the treatment with antibiotics. Following endotoxemia, however, no differences were noted in neutrophil influx, cytokine production, coagulation activation, endothelial activation, or leukocyte responsiveness to multiple Toll-like receptor ligands or clinically relevant bacteria *ex vivo*. This study is reassuring on some levels; however, the relevance of these findings to septic patients with ongoing infection, antimicrobial therapy, physiologic perturbation, and organ failure is not clear. It should be noted, however, that a study of 15 critically ill patients without antibiotic exposure reported significant changes in their microbiome within 6 h of their arrival at the emergency room compared to healthy volunteers.<sup>17</sup> Unfortunately, the concept of “good” and “bad” bacteria is likely overly simplistic as bacteria can alter their own virulence depending on host factors, so the identical bacterial species can be adaptive or maladaptive depending on the clinical situation. Under basal conditions, bacteria rarely express virulence genes. However, in settings of host stress when resources are limited, bacteria can develop both ancestral and newly acquired resistance genes. This could lead to bacterial invasion and, in turn, drive a maladaptive host response.<sup>2</sup> Notably, the timescale in which bacteria can shift their evolutionary trajectories is much shorter (hours) than that of the human host (days to weeks). Thus, the inner microbial world within us has the capacity to adapt to changes faster than the critically ill patient does, which can potentially be devastating if the microbial response is to aggressively attack its host.

An elegant preclinical example of the implications of this was published by Alverdy's group.<sup>18</sup> Both healthy mice and mice that underwent a non-lethal 30% hepatectomy were injected with *Pseudomonas aeruginosa* into the cecum. The bacteria were then withdrawn and injected into the peritoneum of uninjured mice. Animals that received bacteria from healthy mice all survived, yet all animals that received bacteria from mice that had a 30% hepatectomy died. The underlying mechanism is that bacteria injected into mice with a hepatectomy sensed host stress and, in turn, induced virulence factors that subsequently killed the uninjured mouse. As identical bacteria were used in this experiment, this highlights the importance of the host environment in impacting the microbial community, which, in turn, directly impacts the health of the host.

Numerous therapeutic strategies currently exist for manipulating the microbiome in the ICU. These include probiotics, fecal microbial transplant (FMT), and selective decontamination of the digestive tract (SDD). Each of these has shown some promise, yet each also has significant challenges both logistically and intellectually. Probiotics are selective exogenous bacteria given to the host. Meta-analyses and multiple studies have indicated that probiotics are effective at decreasing ventilator-associated pneumonia<sup>19–21</sup> but do not alter length of stay or mortality. A clear limitation to the published works on probiotics is the significant heterogeneity between studies in dose, length, and bacteria given. In addition, most of the studies on probiotics were undertaken prior to our current understanding of the microbiome, implying a more mechanistic design in the future might have greater efficacy. Related strategies are prebiotics, which are non-digestible nutrients that stimulate commensal bacterial growth, and synbiotics (a combination of probiotics and prebiotics). Prebiotics directly regulate host mucosal signaling to alter the response to bacterial infection;<sup>22</sup> however, clinical data are still preliminary.<sup>23</sup> Instead of giving selective bacteria, FMT transfers an entire microbiome from a healthy patient to a diseased one, with the goal of restoring a normal microenvironment. Fecal microbial transplant has proven to be remarkably successful in recurrent *Clostridia difficile* colitis, where a recent meta-analysis of 37 trials shows 92% resolution and a relative risk of 0.23 compared to oral vancomycin,<sup>24</sup> similar to the original landmark study of FMT showing a 93.8% cure rate.<sup>25</sup> However, the usage of FMT in the ICU is currently limited to a small number of case reports.<sup>26</sup> Most ICU patients receive antibiotics that would be expected to immediately alter the transplanted microbial community. In addition, the long-term impact of giving FMT in the ICU is unknown.

Selective decontamination of the digestive tract represents an opposite approach to probiotics or FMT. Rather than

augmenting healthy bacteria or stimulating bacterial growth, SDD seeks to decrease pathogenic bacteria. Selective decontamination of the digestive tract is somewhat of a misnomer as patients receive systemic antibiotics in addition to topical antibiotics. Regardless, this approach has been shown to be very effective with a meta-analysis of nearly 30 high quality trials showing a reduction in mortality with a relative risk of 0.73.<sup>27</sup> However, each of the source studies was undertaken in countries with low basal antimicrobial resistance. Although there is minimal real-world evidence that SDD induces antimicrobial resistance, its use is currently limited to a few countries because of the theoretical concern that SDD could induce resistance.

Conceptually, tricking bacteria into believing a host is healthy could prevent induction of virulence factors. Although this is not feasible at the bedside currently, pre-clinical work suggests this might be a viable strategy in the future. A key determinant of induction of bacterial virulence factors is lack of intraluminal phosphate. Phosphate–polyethylene glycol is a novel preclinical therapeutic that creates local phosphate abundance. When given to septic mice, it prevents development of virulence and importantly improves survival.<sup>28</sup>

Although innumerable insights about the microbiome remain to be discovered, a rapidly evolving understanding of our inner microbial community and its interaction with the host suggests the microbiome could play a central role in the pathophysiology of critical illness. This is conceptually important as a recent review of 51 studies of animals and pediatric and adult ICU patients illustrates multiple ways in which the microbiome can be altered, including metabolomic and proteomic changes, alterations induced by nutrition, and alterations in antibiotic resistance genes.<sup>29</sup> Therapeutic strategies in the future will likely be targeted to restoration of the healthy microbiome, potentially combined with strategies to prevent the development of microbial virulence.

## DISCLOSURE

Approval of the research protocol: N/A.

Informed consent: N/A.

Registry and the registration no. of the study/trial: N/A.

Animal studies: N/A.

Conflict of interest: None declared.

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