

REVIEW ARTICLE

The roles and potential clinical implications of gut microbiome in sepsis

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Abstract

Pronounced dysbiosis in the gut microbiome is common among sepsis patients, resulting in aggravation of the disease. This disturbance not only impacts gut integrity but also initiates localized immune responses that may progress to systemic inflammation. This review explores recent discoveries regarding the dysregulation of the gut microbiome, alterations in gut permeability, and disruptions in intestinal immune responses that occur during sepsis. In addition, we discuss innovative therapeutic strategies, encompassing the impacts of metabolites derived from microbes, the selection of beneficial probiotics, and the utilization of fecal microbiota transplantation in the management of sepsis. Understanding the complexities of the gut microbiome holds the promise of revealing novel strategies that may transform the treatment of sepsis, providing a ray of hope for improved outcomes in critically ill patients.

Keywords: Sepsis; Gut microbiota; Metabolite; Dysbiosis; Immune response

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1. Introduction

Sepsis is a life-threatening disorder caused by the dysregulated host response to infection with a high risk of morbidity and mortality.¹ This syndrome remains a global health concern for the World Health Organization, dramatically influencing resource allocation and causing substantial economic ramifications.^{2,3} In the United States, sepsis incurs nearly \$24 billion in health-care costs annually, surpassing many other diseases, despite constituting a relatively small portion of hospitalization.^{4,5} Recognized through clinical signs and a diverse array of symptoms, sepsis involves a complex interplay of pro- and anti-inflammatory responses, leading to multiorgan dysfunction.¹ Due to the broad spectrum of pathophysiological causes and clinical manifestations, intensivists face challenges pinpointing specific medications for sepsis. Current sepsis treatments mainly

consist of antibiotics and supportive care, with limited options for targeted therapies.⁶ Therefore, comprehending the underlying mechanisms of sepsis and developing novel therapeutic strategies is crucial.

The gut microbiome is a complex ecosystem that plays a crucial and active role in its host. The microbial inhabitants in our gut consist of approximately 100 trillion cells, outnumbering the cells that make up the human body by 10 folds.⁷ The gut microbiome contains about 2 – 4 million genes, showcasing a vast diversity of 100 – 150 times greater than the human genome.^{8,9} The advent of 16S rRNA sequencing and metagenomics enables us to delineate the microbial profile effectively.¹⁰ Through these techniques, the gut bacterial species in healthy volunteers primarily comprise three phyla: *Bacteroides*, *Firmicutes*, and *Actinobacteria*.¹¹ Over the years, evidence has indicated that the gut microbiota may play a crucial role in sepsis.¹² Studies utilizing sequencing methods have elucidated that microbiota imbalance, such as reduced microbial diversity and an abundance of microbial genes, could be impacted by sepsis and *vice versa*.^{13,14}

Protein-calorie malnutrition is prevalent among critically ill patients, often resulting from a combination of factors such as anorexia, diarrhea, and decreased body mass, all influenced by the inflammatory response and hypermetabolism.¹⁵ The dysregulated gut microbiota plays a crucial role in the initiation and progression of sepsis. Patients with sepsis commonly exhibit gastrointestinal dysfunction marked by issues such as altered gut motility and permeability, which can severely hinder digestion and absorption, thereby exacerbating inflammation and contributing to multiorgan failure. Given the gut's crucial function in metabolizing dietary compounds into bioactive molecules, gastrointestinal dysfunction during sepsis can significantly impact the production of protective metabolites.¹⁶

While some aspects of gut dysbiosis have been elucidated, a more comprehensive understanding of how gut microorganisms influence the sepsis process is required. This review aims to deepen our understanding of the intricate relationship between gut microorganisms and sepsis (Figure 1). It specifically focuses on elucidating the changes in gut microorganisms, their functional effects, and the role of microbial-derived metabolites in sepsis, and explores potential therapeutic strategies targeting sepsis through modulation of the gut microbiome.

2. Gut dysbiosis in sepsis

2.1. Sepsis-induced microbiome change

The pathophysiology of sepsis is intricately complex, involving various infection sites and failing organ

systems. Reduced intestinal diversity has been linked to unfavorable outcomes in critically ill patients due to its susceptibility to influences such as antibiotic therapy. A study on septic patients in a Chinese cohort revealed that α -diversity was initially similar between septic and non-septic cases on day 1, but within a week, septic patients exhibited a significant drop in diversity compared to the control group.¹⁷ An investigation into intensive care unit (ICU) patients' fecal microbiota discovered a decreased presence of *Faecalibacterium prausnitzii*, known for its anti-inflammatory function, in both septic and non-septic individuals.¹⁸ Furthermore, a multicenter study involving 155 ICU patients found that septic patients had elevated levels of harmful intestinal microbiota such as *Parabacteroides*, *Fusobacterium*, and *Bilophila* species in perirectal swabs.¹⁹ These microbes are associated with endotoxin production, increased mortality risk, and heightened inflammation, further disrupting metabolic and immune homeostasis.^{20,21}

2.2. Factors contributing to dysbiosis

Critically ill patients are more prone to experiencing disruptions in their intestinal microbiota. While the precise causal mechanisms remain unclear, various factors can contribute to disturbances in the gut microbiome, encompassing extrinsic influences such as antibiotic treatments and intrinsic factors like disease and systemic inflammation.

In ICUs, antibiotics, particularly broad-spectrum ones, are commonly administered to patients suspected of sepsis before bacteriological results are available. A large epidemiological study spanning 500 hospitals revealed that exposure to high-risk antibiotics during hospitalization could disrupt patients' microbiota, potentially heightening the risk of sepsis.²² These high-risk exposures comprised third- or fourth-generation cephalosporins, fluoroquinolones, lincosamides, β -lactam/ β -lactamase inhibitor combinations, oral vancomycin, and carbapenems. Their findings identified that patients exposed to cephalosporins, vancomycin, and β -lactamase inhibitors exhibited a heightened association with the development of sepsis and septic shock.²² Long-term antibiotic administration may lead to the development of resistant gut flora such as vancomycin-resistant *Enterococcus faecium* (VRE)^{23,24} and *Clostridium difficile* infection.²⁵ These alterations and disruptions in the gut microbiota can result in bacterial translocation, facilitated by increased intestinal permeability and compromised gut barrier integrity, potentially exacerbating systemic inflammation. Besides, Mu *et al.*²⁶ described that VRE and *Klebsiella* are predominant in the gut, potentially leading to secondary infections in septic patients following

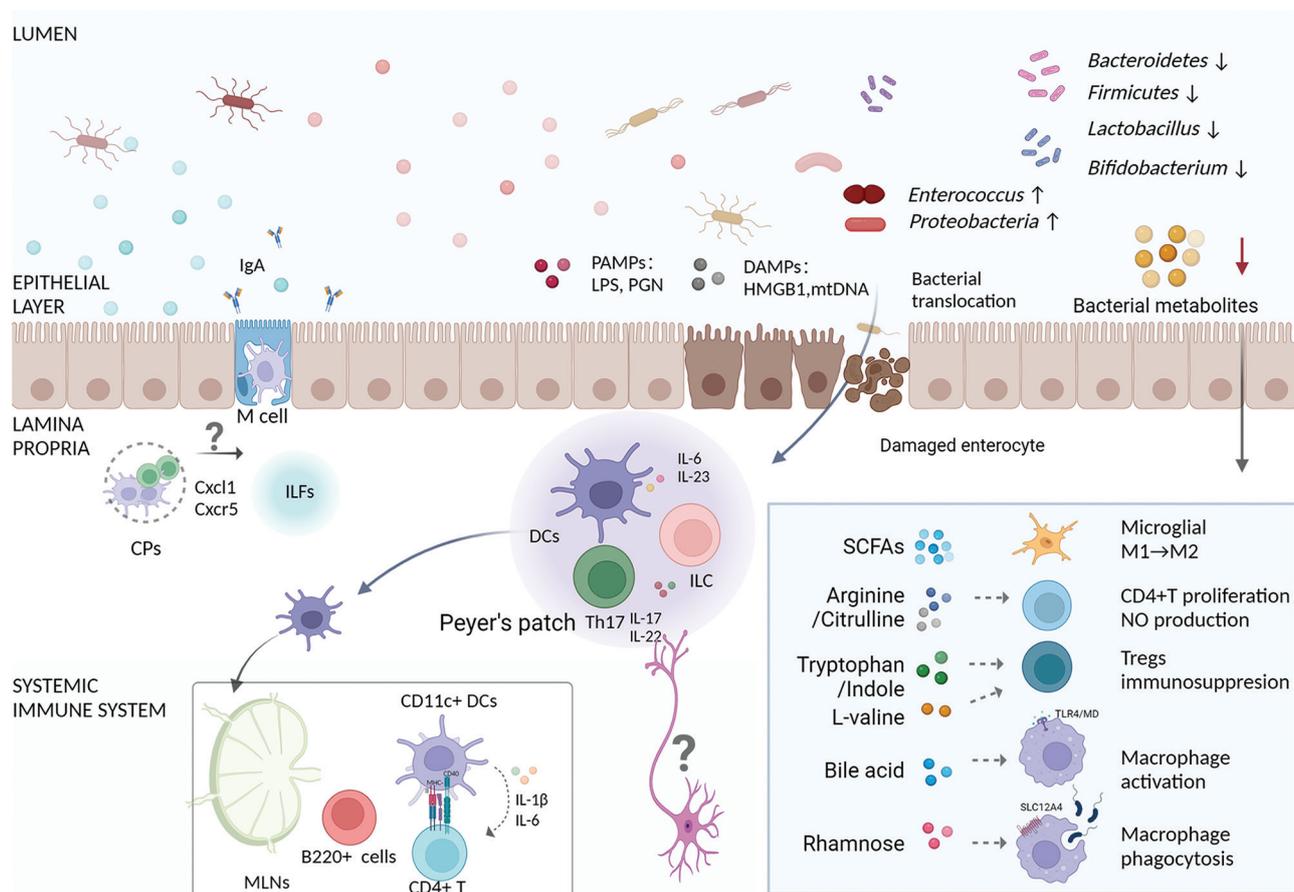


Figure 1. The interplay between gut microbiota, metabolites, and host immune response in sepsis. This schematic illustrates the relationship between the immune response and gut microbiota across the epithelial layer, lamina propria, and systemic immune system. The microbiota is essential for maintaining gut immune function. However, sepsis disrupts the epithelial barrier, leading to an increase in pathogenic bacteria such as *Enterococcus* and *Proteobacteria* and a decrease in beneficial probiotics such as *Lactobacillus* and *Bifidobacterium*. This disruption facilitates the translocation of bacteria and endotoxins, which trigger immune responses locally and systemically. In addition, alterations in gut-derived metabolites can impact the immune system, with supplementation of these metabolites offering protective effects on immune cells, particularly T cells, and macrophages. Within GALT, the specific roles of isolated ILFs differentiation and PPs function, especially in connection with the nervous system, require further investigation. The schematic diagram was created using BioRender.com.

Abbreviations: CPs: Cryptopatches; DAMPs: Damage-associated molecular patterns; DCs: Dendritic cells; GALT: gut-associated lymphoid tissue; HMGB1: High mobility group box 1; ILC: Innate lymphoid cells; ILFs: Isolated lymphoid follicles; LPS: Lipopolysaccharide; M cell: Microfold cell; MLNs: Mesenteric lymph nodes; PAMPs: Pathogen-associated molecular patterns; PGN: Peptidoglycan; PPs: Peyer's patches; SCFAs: Short-chain fatty acids.

broad-spectrum antibiotic therapy. According to these studies, antibiotic usage is believed to be an essential factor influencing the composition of the intestinal microbiota and elevating the risk of sepsis. However, further research is warranted to elucidate the intricate relationship between antibiotics, the gut microbiome, and sepsis, particularly considering the complexity of factors such as dosage and timing of antibiotic administration before or after ICU admission. In addition to antibiotic use, sedatives and analgesics can also perturb the gut microbiome during sepsis. Sedatives and analgesic medications, such as opioids, benzodiazepines, and propofol, are the mainstay in critical care to enhance patient comfort. Notably, morphine, a commonly used analgesic, has been linked

to increased mortality rates in animal models due to the expansion of Gram-positive bacteria such as *Staphylococcus* and *Enterococcus*, leading to bacterial dissemination, upregulation of the proinflammatory cytokine interleukin (IL)-17A, and dysfunction of the gut epithelial barrier.²⁷

Nutrition plays a pivotal role in human health and diseases, with its effects partially mediated through the gut microbiota.²⁸ In contrast to the sepsis samples, the microbial species associated with non-sepsis are frequently observed in non-Westernized populations adhering to traditional lifestyles, characterized by lower fat and phosphatidylcholine intake compared to Western diets. The increased abundance of *Ezakiella*, the butyrate-producing

Megasphaera, and *Prevotella* (especially *Prevotella copri*) could potentially confer a protective effect against sepsis.¹⁹ Similarly, research on a septic animal model treated with cecal ligation and puncture (CLP) indicated that a high-fiber diet could mitigate systemic inflammation and mortality.²⁹ Conversely, a high-fat diet was found to increase mortality and organ damage in the CLP mouse model of sepsis,³⁰ corroborating findings from a U.S. cohort study involving 21,404 participants which indicated that a Southern dietary pattern was linked to a higher risk of sepsis.³¹ However, the specific impact of a high-fat diet on the gut microbiota remains elusive. Further study to deepen understanding of the relationship between diet and microbiota could unveil novel approaches in formulating dietary interventions for septic patients. The Surviving Sepsis Campaign recommends early enteral nutrition for adult patients with sepsis or septic shock due to its potential impact on reducing gastrointestinal complications and influencing gut permeability, inflammation, and immune responses.^{32,33}

3. Host defense mechanisms against sepsis

3.1. Intestine as a barrier in sepsis

The intestinal barrier is a complex structure comprising three essential layers: the luminal layer, mucus layer, and epithelial layer.³⁴ In the luminal layer, commensal gut microbiomes are crucial in inhibiting opportunistic and pathogenic microbial species from colonizing the intestines, especially in ICU patients.¹² The mucus layer acts as a physical barrier that lubricates the contents passing through the intestine and aids in digesting enzymes. This layer thrives in the presence of the microbiome. In addition, it interacts with secretions from Paneth cells and enterocytes, such as lysozyme and immunoglobulin A (IgA), which collectively exert an antibacterial effect.³⁵ Wilmore *et al.*³⁶ revealed the critical role of beneficial microbes in stimulating the production of serum IgA antibodies. These IgA antibodies can help mitigate the severity of polymicrobial sepsis by neutralizing pathogens and impeding their dissemination from the gut into the systemic circulation. Furthermore, the mucus layer is rich in mucins (MUCs) produced by goblet cells. The small intestine has a monolayer of mucus, whereas the large intestine features two substantial layers.³⁷ Among these mucins, MUC2 stands out as the predominant protein responsible for maintaining mucosal homeostasis by restricting the interaction between pathogens and the underlying epithelial layer. In a study with *Muc2*-deficient (*Muc2*^{-/-}) mice, characterized by the absence of an intestinal mucus layer and increased intestinal permeability, it was noted that the efficacy of splenic macrophages in erythrophagocytosis decreased when recognizing

senescent red blood cells during lipopolysaccharide-induced inflammation. Furthermore, these mice exhibited reduced immune apoptosis and an elevated presence of fecal IgA+ bacteria, which potentially contribute to intestinal inflammation.³⁸ This finding illustrated how impaired intestinal permeability can promote bacterial growth and increase the susceptibility to sepsis.

Within the intricate third layer are intestinal epithelial cells, comprising enterocytes, goblet cells, stem cells, neuroendocrine cells, and Paneth cells. Enterocytes, the predominant cell type among intestinal epithelial cells, are responsible for nutrient absorption and immunoglobulin secretion. They facilitate cell–cell communication through structures such as tight junctions, adherent junctions, and gap junctions. Paneth cells, primarily found in the small intestine, can activate toll-like receptors through direct contact with bacteria.³⁹ Generating microbial cell wall components such as pathogen-associated molecular patterns such as lipopolysaccharide and peptidoglycan can trigger excessive immune activation. In addition, damage-associated molecular patterns originating from apoptotic intestinal epithelial cells, such as HMGB1 and mtDNA, can elevate adhesion molecules on the intestinal endothelium, leading to the recruitment of neutrophils and macrophages.⁴⁰ The migration of these immune cells to the gut can induce systemic inflammation characterized by the release of proinflammatory cytokines through MAPK/NF- κ B pathways. This cascade can increase intestinal permeability, exacerbating gut barrier dysfunction by modulating tight junction proteins.⁴¹ The redistribution of the tight junction proteins may facilitate the translocation of local bacteria from the gut lumen.⁴² One such protein, claudin-2, which forms paracellular cation and water channels, is selectively upregulated in septic patients. Deletion of claudin-2 in mice was found to protect against sepsis-induced pore pathway permeability, reducing IL-17 production, T-cell activation, and intestinal damage. This leads to decreased numbers of neutrophils, macrophages, dendritic cells (DCs), and bacteria in the peritoneal fluid of mice. Consequently, claudin-2 deletion significantly improves survival in sepsis.⁴³ By targeting claudin-2, interventions could modulate the microbiome composition and function, offering new avenues for sepsis management and treatment strategies.

3.2. Intestinal immune responses in sepsis

While bacteria and endotoxins may not directly enter circulation, they have the potential to trigger immune responses within the local gut-associated lymphoid tissue (GALT), leading to systemic inflammation. GALT, functioning as a secondary lymphatic organ, protects

the host from invasions within the gut.⁴⁴ Furthermore, mesenteric lymph nodes (MLN), Peyer's patches (PPs), and smaller isolated lymphoid follicles (ILF) are critical components of this complex immune system network.⁴⁵ MLNs are the most prominent lymph nodes in the body, comprising both cortex and medulla.⁴⁶ During sepsis, circulating lymphocytes migrate to the T-cell zone of MLNs, where DCs then present antigens to the T-cells.⁴⁷ Research by Darkwah *et al.*³⁹ revealed a significant increase in CD4 T-cell proliferation by mucosal MLN DCs in the CLP septic model compared to systemic DCs from the spleen, indicating a gut-derived pathway to systemic circulation triggered by bacterial translocation. In addition, O'Boyle *et al.*⁴⁸ identified similarities between organisms in the MLN and the pathogens responsible for sepsis in surgical patients, lending credence to the gut-origin hypothesis for sepsis onset.

PPs are distributed throughout the small intestine, with the highest concentration typically found in the ileum. They comprise lymphoid follicles characterized by a germinal center, subepithelial dome, and follicle-associated epithelium.⁴⁹ The germinal center is densely populated with proliferating B lymphocytes, DCs, and macrophages. In contrast, the subepithelial dome contains a mix of B and T lymphocytes, along with DCs and macrophages. PPs have the unique ability to sample luminal antigens by crossing the epithelial barrier through specialized microfold cells that secrete macromolecules.⁵⁰ The primary role of PPs lies in their communication with the enteric nervous system, thereby contributing to the microbiota-gut-brain axis.⁵¹ Under normal conditions, DCs within PPs detect mucosa-associated bacteria, triggering the production of IL-6 and IL-23, which in turn regulate IL-17 and IL-22 levels in T cells and innate lymphoid cells. Nonetheless, evidence regarding the immune function of PPs in sepsis remains limited.⁵² Schulz *et al.*⁵³ reported that *Salmonella* infection triggered the hypertrophy of PPs and identified the IFNAR/CD69/S1PR1 axis, which facilitates the lymphocyte egress during infection. Conversely, Fan *et al.*⁵⁴ demonstrated reduced PPs cell yield and CD4+T cell count in the CLP model.

ILFs, a specialized type of tertiary lymphoid organs, are notably smaller than PPs and feature a microfold-cell surface epithelium. Despite their significance, there is limited understanding of ILFs in the context of sepsis. These structures typically develop in response to microbial antigens and dietary components in healthy individuals, yet in certain pathological conditions, such as trauma, infection, or other irritations, their formation can be triggered.⁵⁵ ILFs are characterized by a sparse population of T cells and lack distinct T-cell

zones. In germ-free mice, ILFs can be supplanted by a majority of Lin⁻ c-kit⁺ IL-7R α ⁺ ROR γ t⁺ cells known as cryptopatches, which, upon interaction with commensal bacteria, initiate the development of ILFs.⁵⁶ Deficiencies in CXCL13, CXCR5, or ROR γ t may result in the failure of cryptopatches from maturing into ILFs.⁵⁷ Recently, Wu *et al.*⁵⁵ shed light on the connection between the complement system and gut immunity by identifying the presence of C3-expressing cells within ILFs. Studies have shown that sepsis can reduce the quantity and size of lymphoid follicles.⁵⁸ Notably, patients with sepsis exhibit a decrease in B-cell areas and lymphoid follicle counts compared to trauma patients without sepsis. This depletion is particularly pronounced in patients with prolonged septic episodes.⁵⁹ The development of ILFs necessitates lymphoid-inducer cells capable of secreting IL-17 and IL-22, both components of the Th17 signature. In sepsis, IL-17-producing cells such as Th17 and $\gamma\delta$ T17 cells are recognized for their pro-inflammatory nature and their potential role in exacerbating sepsis-related conditions. These cells may infiltrate the brain, leading to sepsis-associated encephalopathy,⁶⁰ or migrate to the lungs, worsening sepsis-induced acute lung injury.⁶¹ These observations highlight a plausible link between sepsis and the development of ILFs.

4. New strategies for treatment of sepsis

4.1. Probiotics

Recent studies have revealed that specific microbial supplements hold the potential to bolster immune responses and alleviate the severity of sepsis. The PRIMAL clinical trial, for instance, has provided evidence that probiotics *Bifidobacterium* and *Lactobacillus* can effectively ameliorate gut dysbiosis in preterm infants, potentially lowering the risk of severe conditions such as sepsis and necrotizing enterocolitis.⁶² In a separate randomized clinical trial investigating probiotic effects on cytokine levels in children with severe sepsis, a notable decrease in pro-inflammatory cytokines and an increase in anti-inflammatory cytokines were observed. This trial involved the administration of a combination of four *Lactobacillus* strains (*Lactobacillus paracasei*, *L. plantarum*, *L. acidophilus*, and *L. delbrueckii*), three *Bifidobacterium* strains (*Bifidobacterium longum*, *B. infantis*, and *B. breve*), and *Streptococcus*.⁶³ Furthermore, Xie *et al.*⁶⁴ discovered that supplementation with the emerging probiotic *Akkermansia muciniphila*, along with its supernatant, can reduce sepsis-induced mortality in a CLP model. In this study, they identified a novel tripeptide, Arg-Lys-His (RKH), as an endogenous antagonist for Toll-like receptor 4 (TLR4). They also highlighted the increasing significance of *Candida albicans* and its derivative metabolite

phenylpyruvate in boosting macrophage bactericidal activity and reducing multiple organ dysfunction syndrome for patients with bacterial sepsis.⁶⁵ In addition, another research group revealed that a reduced abundance of *Parabacteroides* during pregnancy could exacerbate inflammation and worsen sepsis outcomes. Treatment with *Parabacteroides merdae* and its metabolites, particularly formononetin, can protect against septic inflammation by inhibiting macrophage pyroptosis.⁶⁶ Together, probiotics hold promise as a complementary approach to managing sepsis, offering a novel avenue for therapeutic intervention in this critical condition.

4.2. Prebiotics

Prebiotics are non-digestible compounds that can be selectively metabolized by gut microorganisms, providing significant benefits to the host.⁶⁷ While certain non-carbohydrate compounds such as polyphenols and polyunsaturated fatty acids can function as prebiotics, most prebiotics are carbohydrate-based.⁶⁸ Low-molecular-weight carbohydrates are efficiently converted by bacteria. Key examples include fructans (such as fructooligosaccharides and inulin [FOS]) and galactans (such as galactooligosaccharides [GOS]), which promote the growth of beneficial bacteria such as *Lactobacillus* and *Bifidobacterium*.⁶⁹ Clinical studies have demonstrated the potential of prebiotics such as inulin to improve inflammation in conditions such as ulcerative colitis by enhancing the abundance of butyrate-producing *Firmicutes*.⁷⁰ In addition, inulin has been shown to modulate gut microbiota, including *Bacteroides* and *Parabacteroides*, to suppress diet-induced non-alcoholic steatohepatitis.⁷¹ FOS and GOS have similarly demonstrated their ability to regulate inflammatory responses, with clinical trials showing that GOS combined with *Bifidobacterium* improves intestinal barrier function.^{72,73} In addition, other simple carbohydrates such as lactulose also showed a protective effect on intestinal epithelium against the colonization of *Klebsiella pneumoniae*.⁷⁴ Despite these promising findings, the use of prebiotics in sepsis remains limited.

Several challenges contribute to the scarcity of research on prebiotics in sepsis. One key issue is impaired gastrointestinal function, including reduced gut motility and disrupted nutrient absorption, which limit the effectiveness of orally administered prebiotics. Furthermore, many ICU patients rely on parenteral or enteral nutrition, where prebiotics may not be well tolerated or easily incorporated. There is also concern that prebiotics stimulating bacterial growth may increase bacterial translocation and worsen systemic infections. While prebiotics show potential in regulating inflammation and

improving gut health in various diseases, their application in sepsis and critically ill patients in ICU is underexplored.

4.3. Postbiotics

4.3.1. Short-chain fatty acids (SCFAs)

The gut microbiota metabolites are essential for maintaining the fundamental functions of the host in a healthy state. Disruptions in the production of these metabolites can lead to a range of diseases, including metabolic disorders, cardiovascular issues, and gastrointestinal ailments. SCFAs are metabolites produced by the gut microbiome through the fermentation of dietary fibers. These SCFAs, primarily acetate, propionate, and butyrate, play a crucial role in communicating between the gut and the immune system.⁷⁵ Acetate is the most abundant SCFA, produced extensively by bacteria such as *Prevotella* spp., *Bifidobacterium* spp., and *Akkermansia muciniphila*.⁷⁶ Acetate has been shown to regulate immune responses in various disease contexts, including colitis⁷⁷ and arthritis.⁷⁸ Notably, acetate can also modulate the brain's immune system, as demonstrated by its ability to influence microglia during neurodegeneration.⁷⁹ The depletion of SCFAs due to antibiotic disruption of the gut microbiome can lead to hyperresponsive macrophages, a condition that disturbs gut immune homeostasis. Significantly, the supplementation of butyrate alters the activation of these macrophages, restoring a more balanced immune response.⁸⁰ In addition, broad-spectrum antibiotics have been found to promote the colonization of invasive fungi by decreasing SCFA-producing *Clostridium* species.⁸¹ In the context of sepsis, SCFAs have been shown to affect sepsis-induced encephalopathy⁸² by protecting cognitive function and altering the polarization of microglia.⁸³ These studies highlight the critical role of SCFAs in maintaining gut-immune communication and modulating immune responses.

4.3.2. Amino acids

Bacteria in the gut can produce both essential and non-essential amino acids, including glutamine, arginine, and tryptophan. These amino acids contribute to a variety of physiological processes, such as immune regulation, neurotransmitter synthesis, and gut barrier maintenance. However, the composition and function of these amino acid-producing bacteria can be significantly altered during and after sepsis or other infectious events. These disruptions to the gut microbiome can lead to dysregulation in amino acid metabolism.⁸⁴

(A) Glutamine

Glutamine, produced by gut bacteria such as *Bacteroides* and *Clostridium*, contributes to the proliferation of intestinal

cells and maintains the integrity of the gut barrier.⁸⁵ Wu *et al.*⁸⁶ demonstrated that glutamine is particularly important in preserving the intestinal mucus barrier in a mouse model of burn sepsis. Specifically, the researchers found that glutamine promotes the O-GlcNAcylation of the glucose-6-phosphate dehydrogenase (G6PD). This modification enhances the intestinal cells' antioxidant defenses, helping protect them from oxidative stress. At the same time, glutamine inhibits the S-glutathionylation of the protein AGR2, essential for mucus production. By supporting antioxidant mechanisms and mucus production, these glutamine-mediated effects help preserve the integrity of the gut barrier. This, in turn, reduces the risk of bacterial translocation and the development of systemic complications during sepsis.

(B) Arginine

Arginine is a semi-essential amino acid in various physiological processes, including protein synthesis, nitric oxide production, and immune function.⁸⁷ In the gut, arginine is produced by the enterocytes and gut microbiota. During sepsis, the body's demand for arginine increases significantly due to its involvement in immune response modulation, vascular regulation, and tissue repair. However, sepsis-induced dysbiosis can alter the gut microbiota responsible for arginine production, leading to complications in arginine metabolism and availability.⁸⁸ Certain gut bacteria, including *Lactobacillus* and *Bifidobacterium* species, decrease in abundance after the onset of sepsis, and these bacteria are involved in the synthesis of arginine.⁸⁹ These probiotic bacteria can convert the amino acid ornithine into citrulline, which is further converted into arginine through the urea cycle. Clinical trials have explored the potential benefits of arginine infusion in severe sepsis, focusing on its effects on microcirculation and metabolic function.⁹⁰ One randomized controlled trial demonstrated that arginine supplementation could improve microvascular perfusion and support metabolic recovery in septic patients.⁹¹ Furthermore, intravenous administration of arginine has been shown to benefit CD4+ T-cell homeostasis and attenuate liver inflammation in a mouse model of polymicrobial sepsis, thereby boosting T-cell proliferation and function.⁹⁰ Arginine's role in enhancing nitric oxide production helps to improve blood flow and reduce lactic acidosis, which is crucial in managing septic shock. However, concerns remain regarding the balance between the beneficial vasodilatory effects of arginine and the potential risk of exacerbating hypotension in unstable septic patients. These studies highlight the need for careful patient selection and dosing to maximize arginine's therapeutic potential in treating sepsis.⁹² Investigating how arginine supplementation might

restore gut microbiota balance and improve outcomes in septic patients could provide valuable insights into new therapeutic approaches.

(C) Citrulline

Citrulline, a precursor of arginine, has the potential to enhance vasodilation and increase blood flow by stimulating nitric oxide production. Numerous clinical trials have investigated the impact of citrulline on improving the exercise performance of athletes.^{93,94} It has also been reported to support gut health by improving intestinal barrier integrity and modulating intestinal inflammation and can be synthesized by beneficial bacteria such as *Lactobacillus*.⁹⁵ A study conducted by Wang *et al.*⁸⁸ revealed that *Lachnospiraceae* can produce L-citrulline, which, when converted into L-arginine, influences bone mechanical adaptations. During sepsis, especially in ICU patients, citrulline levels often decrease due to gut barrier dysfunction.⁹⁶ The damage induced by sepsis compromises the integrity of the gut lining, impairing the ability of enterocytes to generate citrulline. This reduction is closely linked to increased gut permeability, bacterial translocation, and systemic inflammation, all of which exacerbate the severity of sepsis. Therefore, citrulline levels can serve as a biomarker for identifying gut barrier failure and predicting the overall prognosis of septic patients. Citrulline supplementation in sepsis presents both benefits and risks, acting as a double-edged sword. Research by Asgeirsson *et al.*⁹⁷ has identified citrulline as an anti-inflammatory agent. Moreover, Reizine *et al.*⁹⁸ have demonstrated that enteral citrulline administration can help alleviate sepsis-induced T-cell mitochondrial dysfunction. By restoring arginine levels and promoting nitric oxide production, citrulline boosts T-cell functionality, enhancing immune responses and reducing the severity of sepsis. These findings imply that citrulline may serve as a valuable therapeutic intervention in safeguarding immune function during sepsis. On the contrary, a separate study revealed that citrulline supplementation aggravated sepsis severity in infected preterm piglets.⁹⁹ The early administration of citrulline-induced immunosuppression, possibly attributed to excessive nitric oxide production, exacerbates outcomes. This underscores the dangers of immune overmodulation and emphasizes the importance of timing and context in citrulline supplementation. Nevertheless, two clinical trials propose monitoring citrulline levels in septic patients as a potential biomarker for predicting the development of acute respiratory distress syndrome¹⁰⁰ and overall prognosis.¹⁰¹ A deficiency in citrulline reflects gut barrier dysfunction, which is closely linked to the severity of sepsis and its associated complications. Future investigations could establish citrulline monitoring as a standard protocol for managing septic patients.

(D) Tryptophan

Tryptophan, an aromatic amino acid frequently employed in fluorescence dyes,¹⁰² acts as a precursor for several essential metabolic pathways, particularly the kynurenine pathway, which holds substantial importance in immune regulation during sepsis. Xia *et al.*¹⁰³ uncovered the role of tryptophan metabolism in the pathophysiology of melioidosis induced by the Gram-negative bacterium *Burkholderia pseudomallei*, which results in pneumonia and sepsis. Employing a comprehensive metabolic approach, their research unveiled elevated kynurenine levels and increased indoleamine 2,3-dioxygenase activity, resulting in the enhanced conversion of tryptophan into kynurenine. This process hampers T-cell proliferation and function, leading to a state of “immune paralysis” commonly observed in the advanced stages of sepsis. This immune suppression raises the vulnerability to secondary infections, thereby complicating patient outcomes.

4.3.3. Other metabolites

Alongside the metabolites mentioned above, scientists are continuously discovering novel compounds that play pivotal roles in the progression of sepsis. Indole, a bioactive compound produced by various bacteria, including *Clostridium* and *Lactobacillus* species, maintains the integrity of the gut barrier.¹⁰⁴ Through its actions in promoting mucus secretion and tight junction assembly in the intestine, indole protects the gut lining against bacterial translocation. Moreover, indole and its derivatives, such as indole-3-acetic acid and indole-3-aldehyde, function as ligands for the aryl hydrocarbon receptor, a transcription factor involved in modulating immune responses. Activation of aryl hydrocarbon receptors by these metabolites not only dampens the production of proinflammatory cytokines but also suppresses the differentiation of CD4⁺ regulatory T cells (Tregs).¹⁰⁵ Thus, indole-derived metabolites hold promise for potentially improving outcomes in conditions such as sepsis, where the need to control excessive inflammation while averting immune suppression is paramount.

Li *et al.*¹⁰⁶ unveiled the potential pathophysiological role of gut-derived rhamnose in enhancing macrophage phagocytic activity through its interaction with the SLC12A4 protein, a vital element in the host's defense against polymicrobial sepsis. Other bioactive metabolites such as L-valine have shown promise in preserving intestinal barrier integrity, with findings indicating a negative correlation with APACHE-II and SOFA scores, critical indicators of sepsis severity.¹⁰⁷ Moreover, the secondary bile acid hyodeoxycholic acid (HDCA), derived from primary bile acids by gut microbiota, could mitigate

systemic inflammatory responses post-sepsis by curbing the overactivation of inflammatory macrophages.¹⁰⁸ A positive relationship between HDCA levels and the *Eubacterium* abundance was identified, proposing that supplementing this bacterium could enhance HDCA production, potentially aiding in sepsis management.

4.4. Fecal microbiota transplant (FMT)

FMT presents an innovative approach for tackling the gut dysbiosis linked to sepsis, potentially rebalancing the intestinal microbial ecosystem, safeguarding against intestinal damage, and modulating immune responses. A study reported that FMT could mitigate the immunosuppressive effects of pathogens by restoring normal butyrate levels, exerting impacts beyond the intestine.¹⁰⁹ However, several studies have cautioned about severe complications associated with FMT, including the risk of bacteremia. A case report by DeFilipp *et al.*¹¹⁰ documented instances of *Escherichia coli* bacteremia in two patients post-FMT, which resulted in the unfortunate death of one patient. While FMT holds promise as a therapeutic avenue in sepsis management, comprehensive research is imperative to thoroughly grasp its efficacy and safety profile in clinical settings.

5. Conclusion

Recent advances in metagenomics and metabolomics have markedly enriched our comprehension of the pivotal role the gut microbiome plays in the context of sepsis. This intricate microbial community serves as a cornerstone in regulating host functions, and disruptions within it, known as gut dysbiosis, exert a profound influence on the initiation and progression of sepsis. The intricate interplay between the gut and the immune system presents novel avenues for mitigating organ injury triggered by sepsis, with strategies such as fortifying gut barrier integrity, which demonstrates promise in curtailing bacterial translocation. Following the depletion of gut flora post-sepsis, supplementation with tailored probiotic formulations emerges as a potential avenue for reinstating microbial equilibrium. In addition, the utilization of metabolites synthesized by these beneficial microbes, including SCFAs and essential amino acids, in the formulation of specialized nutritional supplements holds promise in bolstering the recovery of critically ill patients. Interventions aimed at modulating gut microbiomes, such as FMT, exhibit potential for patients grappling with dysbiosis. However, the optimal patient selection, timing of intervention, and administration protocols for FMT warrant further investigation. In summation, a more comprehensive understanding of the gut microbiome harbors the potential to unveil innovative strategies that

could revolutionize the management of sepsis, thereby offering a beacon of hope for enhanced outcomes among critically ill individuals.

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Conflict of interest

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Not applicable.

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