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Review: Microbial Therapeutics for Liver Disease

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Abstract

The human gut contains many microorganisms, including bacteria, fungi, viruses, and archaea. Patients with liver disorders have altered intestinal flora and disrupted gut barriers. The role of the gut microbiota in the pathophysiology of many liver disorders is apparent from preclinical models and clinical studies. High-quality studies showed that people with acute or chronic liver disorders of various etiologies, such as non–alcohol- and alcohol-related liver disease, chronic hepatitis virus infection, chronic cholestatic liver disease, and liver cirrhosis and related complications, have less diverse gut flora and associated perturbed microbial functional metabolism. In this review, we discuss unique therapeutic strategies for various liver diseases that involve manipulating the gut microbiota using various methods. We provide a summary of the most recent information on untargeted methods for treating liver illnesses, such as probiotics, prebiotics, and postbiotics, fecal microbiota transplantation, and precision microbiome-centered treatments (e.g., engineered microbes). Recent research suggests that altering the gut microbiota in various ways might slow the onset of liver disease and lessen the associated clinical complications. Growing evidence suggests that antimicrobial therapy with rifaximin can beneficially alter the gut microbiome to reduce hepatic encephalopathy, portal hypertension, and systemic inflammation in decompensated cirrhosis. At the same time, a healthy donor stool transplant improves transplant-free survival in severe alcohol-associated hepatitis, prevents hepatic encephalopathy, and reduces incident and intercurrent infections and multidrug resistance in decompensated cirrhosis.

Keywords
- gut microbiome
- NAFLD
- sepsis
- hepatic encephalopathy
- hepatocellular carcinoma
- HBV
- HCV
- DILI

Introduction

Culture-free methods utilizing new sequencing technologies and metagenomics have assisted us in identifying trillions of microbes that colonize the human body, most notably and primarily within the gastrointestinal tract. They are collectively referred to as the gut microbiota (GM). The GM's combined genetic information (called the gut microbiome) far outnumbers that of the host. The estimated ratio is 1.3 bacterial cells for every human cell and 1,000 bacterial species with 200 genes per species yielding an estimate of 2,000,000 genes, which is 100 times that of human genes.[1] Chronic diseases such as cardiovascular diseases, diabetes,

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chronic lung disease, chronic liver disease, and cancer, in the context of genetic predisposition, are traditionally thought to be associated with key triggering factors such as dietary habits, alcohol consumption, tobacco use, or other xenobiotic exposures. However, as basic science infrastructure, cutting-edge assessment tools, and in-depth analytical methods have advanced, we have realized that symbiotic GM interactions with the human host play a critical role in maintaining health via homeostatic mechanisms at the cellular, tissue, and organ levels.[2] The presence of triggering factors disrupts the homeostatic relationship, resulting in qualitative and quantitative changes within the GM. This disruption, termed dysbiosis, is linked to several disorders, including acute and chronic liver diseases (►**Fig. 1**). The interaction between the GM and liver is bidirectional because the liver receives 75% of its blood supply from the intestines via the portal vein.[3,4] Moreover, the biliary tree releases various metabolites, particularly bile acids, which influence various microbial functions. Similarly, the functional metabolites produced by the GM act at the local, regional, and systemic levels in the promotion of health or the causation or disease progression, depending on the enigmatic "healthy microbiota," which requires clarification in assessment, measurement, and definition.[5] Multiple malignancies, inflammatory bowel diseases, obesity, alcohol-associated and nonalcoholic hepatic steatosis, diabetes mellitus, and functional gastrointestinal problems have been linked to intestinal dysbiosis. Numerous studies have also revealed the critical role that gut dysbiosis plays in the onset and progression of chronic liver disease due to various etiologies.[6]

This review aims to outline how the GM and liver interact in the context of various liver diseases, with a focus on therapeutic strategies that help modulate GM for the treatment of alcohol-associated liver disease (ALD), metabolic dysfunction–associated fatty liver disease (MAFLD), chronic cholestatic liver diseases, hepatocellular carcinoma (HCC) and cirrhosis, and its complications, and acute and chronic liver failure (ACLF).

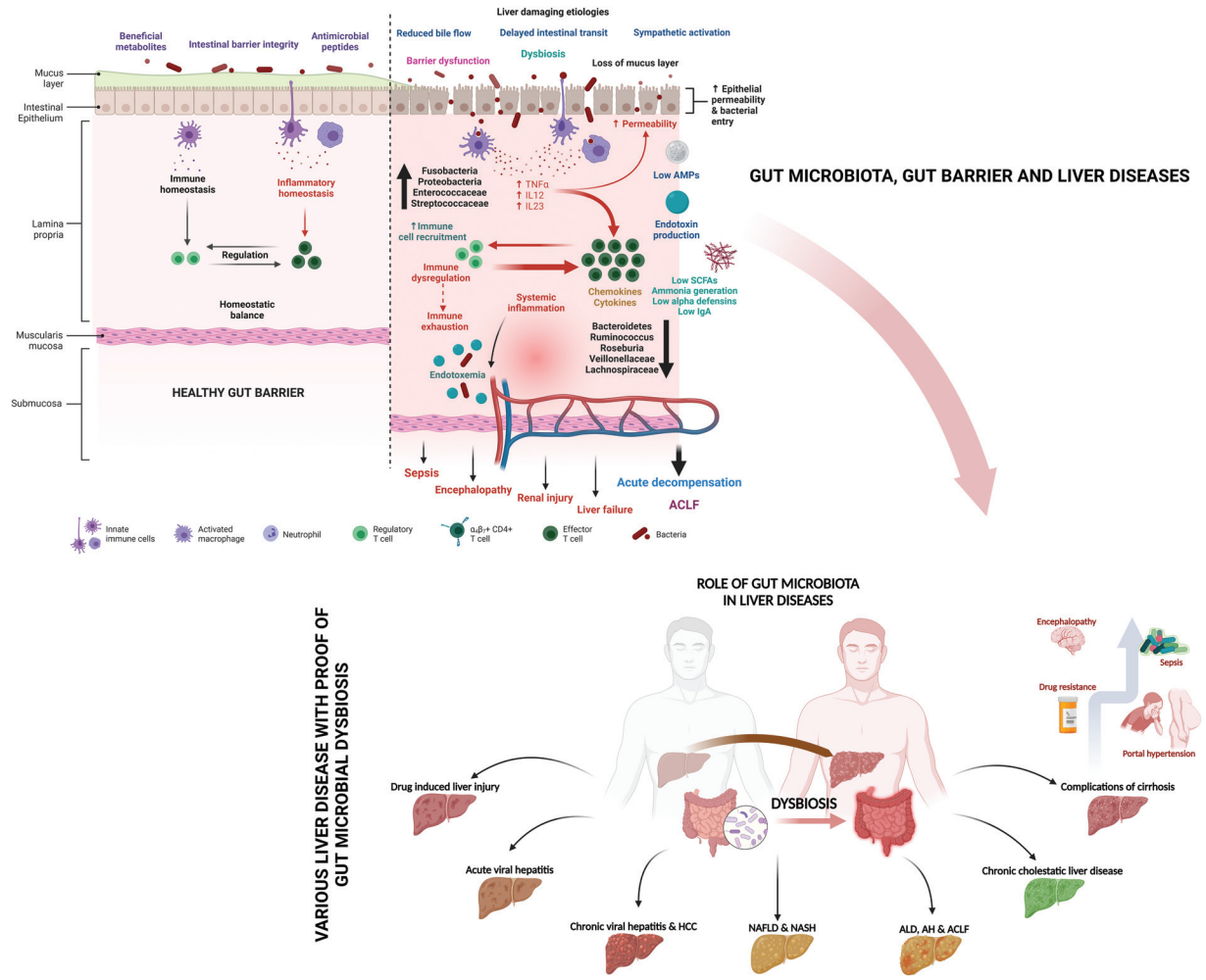

Fig. 1 Healthy intestinal barrier and its disruption due to various etiologies related to liver disease development. Liver injury leads to reduced bile flow, delayed intestinal transit, gut mucosal dysfunction, and increased intestinal permeability. The associated gut dysbiosis modifies the local and systemic inflammatory milieu leading to perturbed metabolism that affects the host by driving the progression of liver disease and resulting in associated complications. Various liver disease conditions are associated with intestinal dysbiosis. ACLF, acute-on-chronic liver failure; AH, alcohol-associated hepatitis; ALD, alcohol-associated liver disease; HCC, hepatocellular carcinoma; IL, interleukin; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; SCFA, short-chain fatty acid; TNF, tumor necrosis factor.

Role of Gut Microbiota in Liver Disease

Most microorganisms in the human intestinal microbiota belong to 12 distinct bacterial phyla. About 93.5% comprises the phyla Firmicutes (*Roseburia*, *Ruminococcus*, *Clostridium*, *Blautia*, *Enterococcus*, *Faecalibacterium*, *Eubacterium*, *Streptococcus*, and *Lactobacillus*); Proteobacteria (Enterobacteriaceae, including *Escherichia* and *Klebsiella*); and Actinobacteria. There is also a trace of Verrucomicrobia, which is dominated by the bacterium *Akkermansia muciniphila*, and several fungi (mostly *Candida* species), viruses, and bacteriophages.[7,8] These microbial communities' diversity, makeup, and functionality can change depending on genetic, gender-related, metabolic, nutritional, and environmental factors. Alcohol consumption and obesity are two examples of host- and environment-derived factors that influence the intestinal microbiota physiologically. These changes in the microbiota cause dysbiosis, bacterial translocation that increases intestinal permeability, and further impairment of functional metabolisms, such as abnormal bile acid metabolism in the gut lumen. This allows microbial byproducts to enter the liver via the portal venous system and lymphatics, causing local and systemic inflammation and exacerbating gut dysbiosis, promoting the onset or progression of many liver diseases.[9,10] Small intestinal bacterial overgrowth and increased levels of circulating proinflammatory cytokines have been linked to gut dysbiosis, which leads to gut barrier dysfunction, worsening dysbiosis, and disease onset or progression. Low species richness and diversity in dysbiotic microbial communities result in a pathogenic functional profile, triggering several clinical events in people with liver disease progression. Infections, metabolic encephalopathy, and extrahepatic organ failure are linked to clinical syndromes, including acute liver failure (ALF), acute decompensation, or ACLF, and systemic inflammation that worsen over time.[11,12] ►**Fig. 2** depicts an example of various GM interactions and their functional metabolism affecting patients with severe ALD.

Gut Microbiota in Viral Hepatitis

The evolution of acute viral hepatitis (A and E) is linked to bacterial translocation, which causes intestinal inflammation via immune cell dysregulation, barrier malfunction, and the emergence and development of dysbiosis. Various key preclinical studies have shed light on the correlation of GM in acute viral hepatitis. Nonetheless, strong causative links of GM in the natural history of acute viral hepatitis remain largely unknown. In pigs, the beneficial probiotic *Enterococcus faecium* NCIMB 10415 reduced and eliminated enteric hepatitis E viruses. In acute enteric viral infections, the GM can induce homeostatic type I interferon expression from macrophages and plasmacytoid dendritic cells and homeostatic type III interferon expression from intestinal epithelial cells.[13] In patients with acute viral hepatitis E infection, the group with increased interferon-gamma was associated

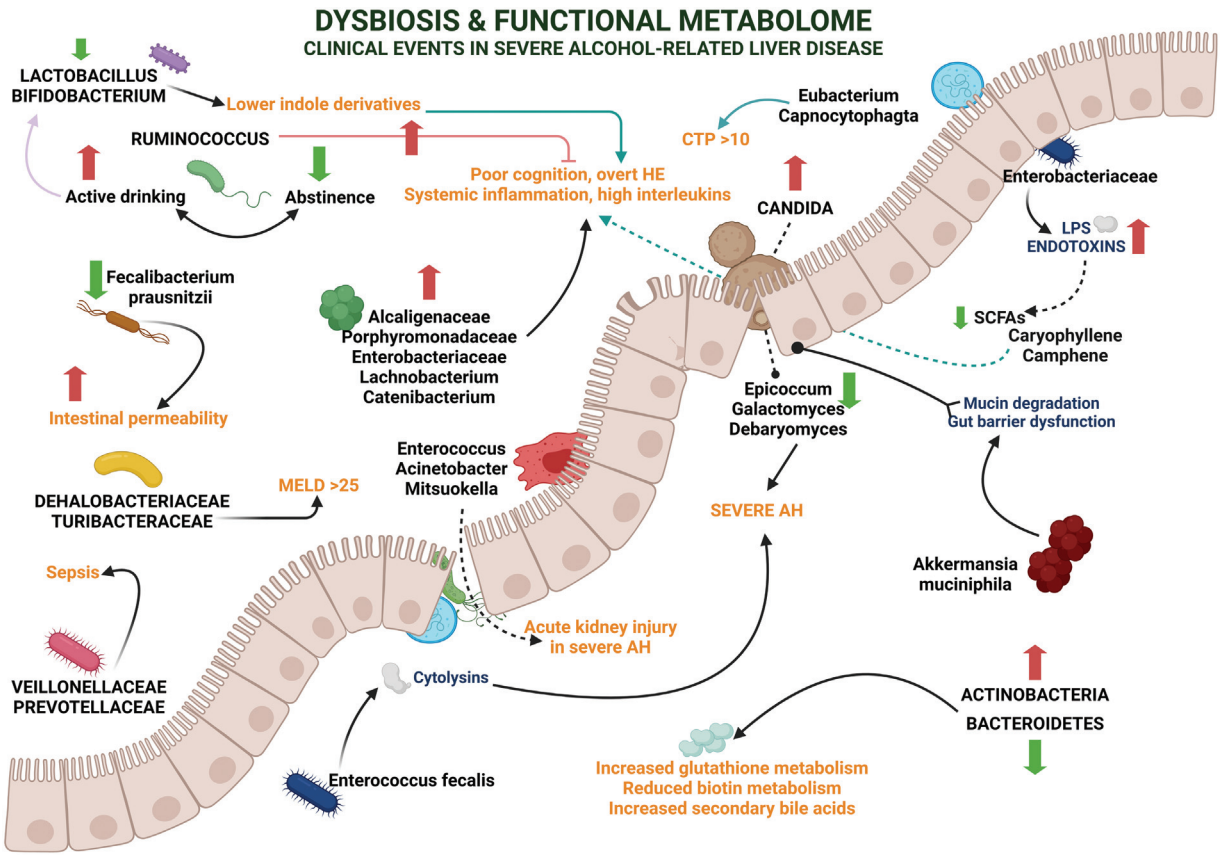

Fig. 2 Intestinal dysbiosis and associated perturbed qualitative and functional metabolism within the bacterial communities associated with various clinical events and liver disease severity in patients with alcohol-associated hepatitis.

with higher relative abundances of Proteobacteria, Gammaproteobacteria, Xanthomonadaceae, and Enterobacteriaceae. A higher abundance of Gammaproteobacteria correlated with serum alanine transaminase, total bilirubin levels, and the severity of acute hepatitis.[14] Numerous studies have shown that people with chronic hepatitis B or C, regardless of cirrhosis, have significantly different GM compositions with reduced species diversity resulting in a decreased capacity to respond to changes caused by local and systemic inflammation. The composition and function of commensal microbiota influence viral replication, interactions between viruses and their hosts, and the chronic phase of hepatitis B and C viral infections. Pathogenic genera, including *Escherichia coli*, Enterobacteriaceae, *Enterococcus faecalis*, and *Faecalibacterium prausnitzii*, can directly alter the composition of native bacteria in viral hepatitis B, causing a decrease in the abundance of *Lactobacillus*, *Pediococcus*, *Weissella*, and *Leuconostoc*. Hepatitis B virus (HBV) infection was linked to a gradual decrease in butyrate-producing bacteria and an increase in endotoxin-producing taxa. By reducing lipopolysaccharide (LPS) release and bacterial translocation, the beneficial phylum Lachnospiraceae contributes significantly to controlling HBV infection.[15] Chronic hepatitis B patients had an excess of the *Anaerostipes* taxon in their gut microbial analysis compared with healthy controls. Researchers discovered that Neisseriaceae positively correlated with serum levels of hepatitis B viral DNA, and the oral microbiota dysbiosis during hepatitis B infection was associated with a yellow coating of the tongue, indicating a decrease in Bacteroidetes but an increase in Proteobacteria.[16]

HCC associated with hepatitis B progresses in a manner significantly influenced by the gut microbiome. Patients with chronic hepatitis B and liver cancer had lower species richness and increased relative abundance of proinflammatory bacterial groups such as those associated with Proteobacteria. Similarly, infection with HBV reduces the population of anti-inflammatory bacteria such as *Prevotella* and *Faecalibacterium*.[17] People in the immune-tolerant or immune-active phase of HBV infection have different GM makeup and several functional metabolites.[18] Four phyla of Firmicutes, Bacteroidetes, Actinobacteria, and Proteobacteria were the most abundant, accounting for 99.72, 99.79, and 99.55% in the healthy controls, immune-tolerant and immune-active phase patients, respectively. Within HBV-infected immune-tolerant and immune-active phase patients, six metabolic pathways were enriched on microbial functional analysis: carbohydrate, amino acid metabolism, lipid metabolism, cofactors, and vitamins metabolism, xenobiotic metabolism biodegradation, and metabolism of terpenoids and polyketides when compared with healthy controls.

According to research on hepatitis B–related ACLF, the relative abundance of the phylum Bacteroidetes was dramatically reduced, and that of potentially pathogenic bacteria such as *Veillonella*, *Streptococcus*, *Enterococcus*, and *Klebsiella* increased, compared with controls. Furthermore, *Veillonella* levels were positively related to blood total bilirubin, whereas Bacteroidetes levels were negatively related to serum α-fetoprotein. *Coprococcus* abundance had a negative correlation with coagulation parameters and hyperbilirubinemia. Furthermore, researchers discovered that the bacterial community composition changed as hepatitis B–related ACLF progressed. Increased *Enterococcus* levels were associated with ACLF development, whereas increased *Faecalibacterium* levels were associated with ACLF regression.[19] In one study, patients with chronic hepatitis C virus infection had higher Enterobacteriaceae and Bacteroidetes and lower Firmicutes. As the illness progressed to cirrhosis and decompensation, these alterations were accompanied by increased LPS levels, indicating gut barrier disruption and microbial translocation. After receiving direct-acting antiviral therapy, nonindigenous and pathogenic taxa, including Enterobacteriaceae, *Staphylococcus*, and *Enterococcus*, decreased in the gut of cirrhotic individuals with hepatitis C. Nonetheless, a recent study found that HCV patients did not have significant GM changes, and HCV eradication with direct-acting antivirals was not associated with significant and beneficial modulation of intestinal microbiota.[20,21] ►**Table 1** depicts the key features associated with GM changes in HBV and HCV infection.

Gut Microbiota in Metabolic Dysfunction–Associated Fatty Liver Disease and Steatohepatitis

Dysbiosis has been linked to the development of cirrhosis, the transition from MAFLD to nonalcoholic steatohepatitis (NASH), and certain clinical manifestations of cirrhosis. Compared with healthy controls, patients with MAFLD had higher levels of Prevotella and *Porphyromonas* spp. and lower levels of Bacteroidetes. Researchers discovered that ethanol-producing *Escherichia* was more common in patients with NASH; they had lower Bacteroidetes, *Faecalibacterium*, *Lactobacillus*, and *Ruminococcus* levels and higher *Clostridium coccoides*, Proteobacteria, and *Escherichia* levels.[22] A high proportion of *Bacteroides* and *Ruminococcus* taxa was associated with higher fibrosis grades or NASH in patients with cirrhosis. Trimethylamine N-oxide, ethanol, and lactate are examples of gut microbiome–derived metabolites that reduce the total bile acid pool, which can influence farnesoid X receptor signaling and the development of MAFLD. Acetate, propionate, and butyrate are short-chain fatty acids (SCFAs) produced by bacterial species that slow down the progression of MAFLD.[23]

Intestinal bacteria play an essential role in the metabolism of dietary choline. Researchers discovered that high-fat diet–fed mice had increased choline degradation by GM, resulting in low plasma phosphatidylcholine levels and lower choline bioavailability, which have been linked to the causation of MAFLD. Choline deficiency is known to cause NASH in animal models. Choline-deficient diets were found to increase the risk of fatty liver, and bacterial Firmicutes, *Erysipelotrichia*, Proteobacteria, and Gammaproteobacteria were linked to choline deficiency–induced fatty liver.[24] A recent study discovered that the composition and function of the GM in patients with MAFLD and progressively increasing liver stiffness were significantly altered. Whole-genome sequencing revealed that the GM composition of patients with a liver stiffness measurement > 7.4 kPa differed significantly from

Table 1 Gut microbiota differences in patients with chronic viral hepatitis B and C

	Increased	Decreased
Hepatitis B		
Chronic HBV infection	Proteobacteria, Actinobacteria, *Bifidobacterium dentium*, Veillonellaceae	Bacteroidetes, Firmicutes, *Bifidobacterium catenulatum* and *B. longum*, Lachnospiraceae, Rikenellaceae, Ruminococcaceae
Cirrhosis (compensated)	Proteobacteria, Actinobacteria	Firmicutes, Bacteroidetes
Cirrhosis (decompensated)	Enterobacteriaceae	*Faecalibacterium prausnitzii*, *Bifidobacterium*, Firmicutes, *Clostridium clusters XI and XIVab*, and Bacteroidetes
Hepatocellular carcinoma	*Proteus*, *Veillonella*, *Barnesiella*, *Ruminococcaceae*, *Prevotella*, *Phascolarctobacterium*, and *Anaerotruncus*	Proteobacteria
Hepatitis C		
Precirrhotic stage	*Streptococcus*, *Veillonella*, *Lactobacillus*, and *Alloprevotella*	*Mitsuokella*, *Vampirovibrio*, *Bilophila*, *Clostridium IV* and *Clostridium XlVb*
Cirrhosis	*Streptococcus*, *Veillonella*, several *Lactobacillus species*, *Alloprevotella*, *Akkermansia*, *Bifidobacterium*, *Escherichia/Shigella*, *Micrococcus*, *Weissella*, and *Haemophilus*	*Mitsuokella*, *Vampirovibrio*, *Bilophila*, *Clostridium IV* and *Clostridium XlVb*
Advanced cirrhosis	*Prevotella*, *Faecalibacterium prausnitzii*, *Acinetobacter*, *Veillonella*	*Firmicutes*

Abbreviation: HBV, hepatitis B virus.

that of the control group.[25] An intriguing Dutch study found that GM ethanol production played a significant role in the pathogenesis of MAFLD, with Lactobacillaceae specifically correlated with postprandial peripheral ethanol concentrations.[26] Preclinical research found that transplanting the GM of lean mice into obese mice caused them to become "lean," which was linked to a significant change in the intestinal microbial composition. Regardless of the donor body mass index, there was no discernible change in the body mass index of recurrent *Clostridium difficile* patients 1 year after a single session of fecal microbiota transplantation (FMT). Nonetheless, compared with autologous microbiota, overweight patients with metabolic syndrome had significantly improved insulin sensitivity (both hepatic and peripheral) after 6 weeks of FMT from lean donors.[27] ►**Table 2** shows the relevant GM changes associated with mild or severe MAFLD.

Gut Microbiota in Alcohol-Associated Liver Disease

The composition, variety, and metabolic function of the GM affect the integrity of the intestinal barrier and systemic inflammation, all of which play a role in the development and progression of ALD. A study of human GM revealed that ALD without cirrhosis was associated with higher Proteobacteria and lower Bacteroidetes. Ruminococcaceae levels were lower in active drinkers' intestines.[28] Patients with ALD and underlying cirrhosis demonstrated worse dysbiosis than those with non-ALD cirrhosis of comparable severity, highlighting the significant contribution of alcohol to

Table 2 Microbiota changes in different studies regarding nonalcoholic fatty liver disease

	Increased	Decreased
NAFLD		
NAFLD vs healthy controls	Proteobacteria Enterobacteriaceae *Escherichia*, *Dorea*, *Peptoniphilus*	Firmicutes Rikenellaceae, Ruminococcaceae *Anaerosporobacter*, *Coprococcus*, *Eubacterium*, *Faecalibacterium*, *Prevotella*, *Clostridium sensu stricto*
NASH vs NAFLD (no-NASH)	Enterobacteriaceae *Shigella*, *Bacteroides*	Firmicutes Prevotellaceae Clostridiaceae *Prevotella*, *Clostridium sensu stricto*

Abbreviations: NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis.

intestinal microbiota dysbiosis. A lower *A. muciniphila* was linked to more severe ALD.[29] Active drinkers with cirrhosis had higher secondary bile acid levels due to more noticeable bacterial metabolism in their feces than nondrinkers. A new study has linked intestinal microbiota to clinical events and treatment outcomes in people with severe ALD. In patients with alcohol-associated hepatitis (AH), *Catenibacterium* and *Lachnobacterium* were linked to hepatic encephalopathy (HE). *Pediococcus* was significantly more common in patients who died during follow-up after failing to respond to corticosteroid treatment.[30,31] Researchers assessed the intestinal virome and fungal communities (mycobiome) in patients with severe ALD in addition to the bacterial population. Patients who drank heavily had alcohol use disorder, developed progressive liver disease, and had significantly higher levels of *Malassezia*.[32,33] Patients with severe AH experienced the most changes in viral diversity. In addition to increased mammalian viruses, including Parvoviridae and Herpesviridae, patients with severe AH had an excess of *Escherichia*-, *Enterobacteria*-, and *Enterococcus*-associated phages (►**Fig. 3**).

Acute Liver Injury and Acute Liver Failure: The Case of Drug-Induced Liver Injury and Autoimmune Hepatitis

Acute viral hepatitis, acute alcohol-induced liver injury, various nonhepatotropic infectious causes, drug-induced liver injury, and autoimmune hepatitis (AIH) are some causes of acute liver injury (ALI). Herbal and dietary supplements are the most common cause of ALI. ALF is a severe symptom of ALI that is clinically defined by jaundice, impaired coagulation function, and HE emergence within 4 weeks of the onset of jaundice. The GM plays an important role in the host's ability to process endogenous and exogenous substances. Acetaminophen accumulates in the liver due to the GM's increased production of *p*-cresol, which reduces acetaminophen's sulfonation.[34] Microbial metabolites, such as 1-phenyl-1,2-propanedione (PPD), produced by *E. coli* and *Citrobacter freundii*, increased acetaminophen's liver toxicity. PPD directly depletes hepatic glutathione and increases the formation of dangerous acetaminophen adducts.[35]

Monoclonal anti-Toll-like receptor 4 antibodies alter the GM, metabolic pathways, and gut barrier function to reduce acetaminophen-induced ALI, indicating that the GM could be a therapeutic target for acetaminophen-induced ALI.[36] Researchers examined rats given the hepatotoxic drug tacrine. They discovered that rats with severe liver damage (strong responders) excreted more total tacrine in their feces. Furthermore, the strong responders had higher levels of two bacteria that promote glucuronidase activity: Bacteroides and Enterobacteriaceae. The vulnerability to tacrine-induced hepatotoxicity was significantly reduced after receiving antibiotic treatment. This well-designed study provided critical evidence for how gut microbial activities influenced the development of drug-related hepatotoxicity.[37] Similar evidence supports the role of specific bacterial genera, namely, *Lactobacillus* and *Bifidobacterium*, as well as their metabolic enzymes, such as alpha-glucosidase and rhamnosidase in the deglycosylation of herbal drug–related compounds such as

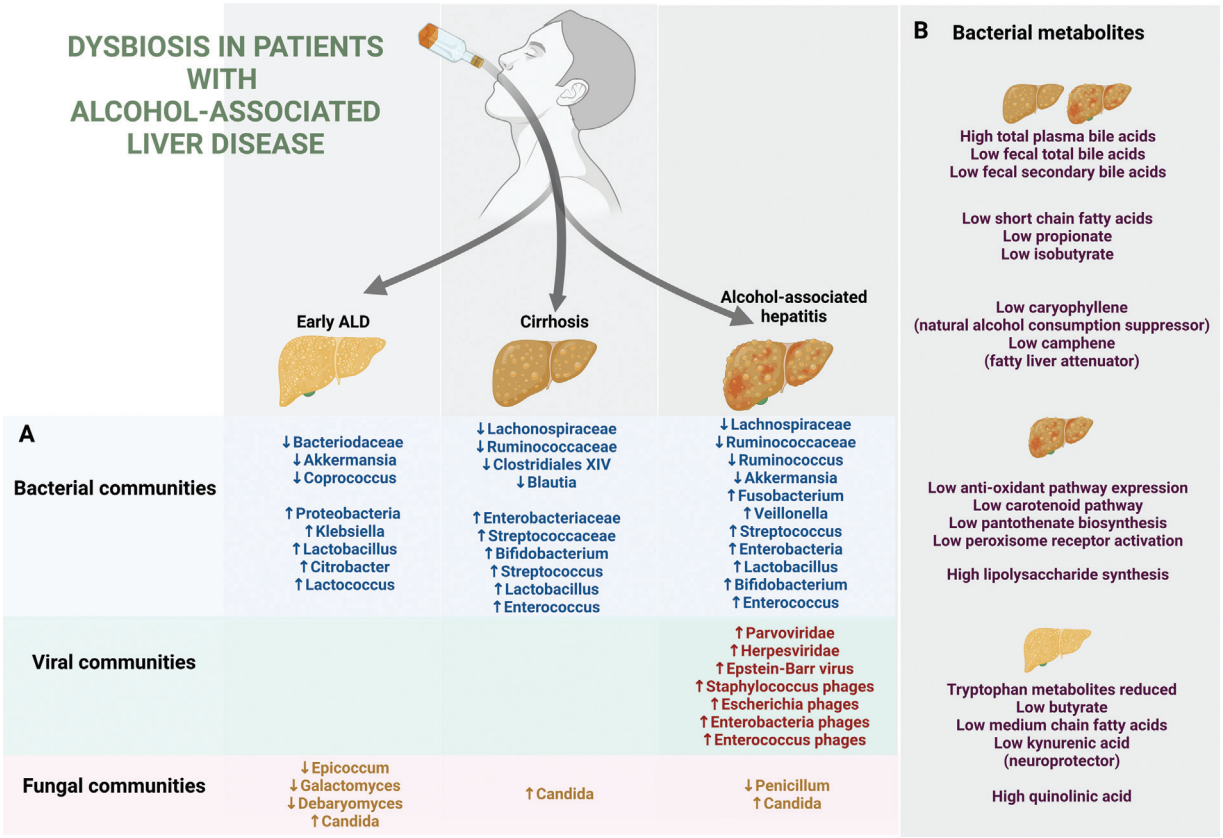

Fig. 3 Various dysbiotic microbial afflictions (within the host gut) associated with stages of alcohol-associated liver disease.

ginsenoside. Similarly, inulin, chicory root, and flaxseeds influence GM by producing SCFAs and influencing healthy gut barrier function.[38] Furthermore, it has been demonstrated that intestinal microbial–derived antigens (glycolipids) played a critical role in activating liver-associated natural killer T cells to mediate concanavalin A–induced severe AIH and liver failure.[39]

The administration of D-galactosamine affected the intestinal anti-inflammatory molecule soyasaponin II, significantly altering the composition and function of gut microbes. By allowing different microbiota-associated molecular patterns to enter local and systemic levels, a decrease in soyasaponin II levels caused hepatocyte damage and damage to the gut mucosal barrier. It was recently demonstrated that pretreatment with the probiotic *Lactobacillus reuteri* improved gut dysbiosis, which improved inflammatory factor transcription and reduced D-galactosamine–induced liver injury. Transplantation of feces from *Saccharomyces boulardii* donor mice reduced the liver damage caused by D-galactosamine.[40] Researchers discovered that the gut microbiome of patients with acute hepatitis associated with AIH who did not receive steroid treatment had less α diversity than healthy controls. Obligate anaerobes were reduced, whereas pathogenic taxa, including *Veillonella*, the taxon most strongly linked to disease, increased and were significantly linked to the disease development. This established a link between compositional and functional changes in the gut microbiome and acute AIH.[39]

Gut Microbiota in Cirrhosis and Associated Complications, Including Primary Liver Cancer

In small animals and humans, considerable research has been conducted to determine the role of GM in the etiology, development, and clinical consequences of chronic liver disease. Preclinical research has shown that the bacterial families Streptococcaceae, Staphylococcaceae, and Lactobacillaceae influence ammonia metabolism, brain function, and systemic inflammation.[41] A comprehensive meta-analysis found a link between chronic liver disease progression dysbiosis and small intestine bacterial overgrowth. This was most prominently demonstrated in ALD-related cirrhosis, where dysbiosis and a shift toward pathogenic bacterial genera such as *Rothia*, *Streptococcus*, and *Shuttleworthia* were linked to increased intestinal permeability, microbial translocation, and progressive steatohepatitis and steatofibrosis in people who continued to drink. Cirrhotic patients have decreased diversity and abundance of beneficial native taxa such as Ruminococcaceae and Lachnospiraceae. The expansion of pathogenic taxa such as Enterobacteriaceae, Staphylococcaceae, and Enterococcaceae is linked to this. Infections such as bacterial peritonitis were linked to lower Firmicutes levels in cirrhosis.[42,43]

Lower levels of indigenous bacterial communities were associated with higher end-stage liver disease scores, whereas higher levels of Bacteroidetes were associated with endotoxemia. Regarding etiology, Bacteroidetes and Firmicutes showed the most significant changes in the GM in alcohol-associated cirrhosis, with a greater drop in the former. However, Firmicutes were found to be less prevalent in cirrhosis caused by other etiologies. The cirrhosis dysbiosis ratio, which measures the degree of dysbiosis, decreases as cirrhosis progresses (inversely proportional).[44] *Streptococcus salivarius* was linked to higher blood ammonia levels and covert HE. Proteobacteria on GM analysis have also been linked to cirrhosis, endotoxemia, and cognition. In patients with cirrhosis, pathogenic taxa such as Porphyromonadaceae, Lactobacillaceae, Staphylococcaceae, and Enterococcaceae were positively connected with functional magnetic spectroscopy brain results, whereas native taxa were negatively correlated. Reduced Bacteroidetes abundance was linked to an increased risk of infection in ALD-induced cirrhosis. Regardless of clinical characteristics or disease severity ratings, dysbiosis on admission was also associated with an increased risk of extrahepatic organ failure, ACLF, and mortality. Next-generation sequencing studies also revealed a link between the progression of cirrhosis and a sharp decline in species diversity and gene expression, most notably in those developing ACLF, which was also linked to an increase in *Enterococcus* and *Peptostreptococcus*. Surprisingly, changes in the GM linked to pathways involved in aminobutyric acid metabolism, endotoxin biosynthesis, and ethanol production predicted 3-month survival in cirrhotic patients who developed ACLF.[45]

Cirrhosis worsens dysbiosis and microbiome profile, exacerbated by repeated hospitalizations, antimicrobials, and proton-pump inhibitors. These factors, especially repeated in-hospital treatment and interventions, promote multidrug resistance and subsequent infection-related organ failure, both of which are associated with poor clinical outcomes. According to a recent study, patients with decompensated cirrhosis, sepsis, and immune exhaustion were more likely to have pathogenic *Corynebacterium* and *Lautropia* genus. Sepsis significantly increased the sulfur relay and LPS production metabolic pathways associated with oxidative stress and endotoxemia. In people without sepsis, protective oxidant mechanisms that boost glutathione were elevated. In patients with interleukin-6 levels $>$ 1,000 pg/dL, pathways of severe LPS-related hyperinflammatory stress, exaggeration of orally prevalent pathogens (*Prevotella*), and sulfur-metabolizing bacteria from the Gammaproteobacteria family were observed. In advanced cirrhosis patients with two or more infection episodes, pathogenic genera associated with immune paralysis were prominent.[46] Furthermore, the GM profile was shown to distinguish between cirrhosis of various etiologies. For example, *Neisseria* and *Gemella* expansion aided in distinguishing primary biliary cholangitis (PBC) from cirrhosis caused by the HBV.[47]

Dysbiosis of the GM can increase gut permeability resulting in microbial translocation and increased hepatic exposure to microbiota metabolites and products. Various oncogenic products subsequently aid in the development of HCC and liver disease progression. Numerous studies have found significant changes in the bacterial population of various cirrhosis etiologies linked to the development of HCC. Patients with early HCC, compared with cirrhosis without HCC, had higher levels of Actinobacteria and lower

levels of Verrucomicrobia.[48] *Gemmiger*, *Parabacteroides*, *Klebsiella*, and *Hemophilus* were significantly more prevalent in stool metagenomics in those with early HCC than without HCC. *Ruminococcus*, *Phascolarctobacterium*, and *Alistipes* levels were lower in HCC patients.[49] In NASH cirrhosis, patients with HCC showed higher levels of *Bacteroides* and Ruminococcaceae in their guts, whereas *Bifidobacterium* was significantly lower. The relative abundance of *Faecalibacterium*, *Ruminococcus*, and *Ruminiclostridium* was higher in HCC caused by hepatitis B and C coinfection. A study showed that the absence of *A. muciniphila* correlated with the abundance of hepatic monocytic myeloid-derived suppressor cells. Simultaneously, its reintroduction restores intestinal barrier function while significantly reducing liver inflammation and fibrosis, which are prerequisites for cancer development.[50,51]

Gut Microbiota in Chronic Cholestatic Liver Diseases

According to research, patients with PBC have altered gut microbiomes. While Enterobacteriaceae, *Klebsiella*, *Hemophilus*, *Veillonella*, *Streptococcus*, and *Lactobacillus* were abundant, Bacteroides were not. Faecalibacterium stool levels were lower in PBC patients who did not respond to ursodeoxycholic acid therapy. Furthermore, primary sclerosing cholangitis (PSC), extensively studied in human and animal models, is linked to gut dysbiosis. Less α diversity increased *Veillonella* and *Clostridium* taxa, and less *Coprococcus* and *Faecalibacterium* abundance was observed in PSC patients. *Escherichia* and *Megasphaera* levels in the stools were higher in PSC patients, but *Prevotella*, *Roseburia*, and *Bacteroides* levels were lower.[52–55]

Microbiota-Based Modulatory Therapies and Their Impact on Various Liver Diseases

Restoring the gut barrier and a healthy (autochthonous) GM is considered one of the main therapeutic targets for several liver diseases. *Klebsiella pneumonia* causes endogenous ethanol production in MAFLD/NASH, cytolysin-producing *E. faecalis* causes severe AH, *Enterococcus gallinarum* causes AIH, and *Veillonella* causes PSC. Microbial modulation or restoration can be accomplished through dietary changes, antibiotics, probiotics, prebiotics, synbiotic supplementation, FMT, and phage treatments. In the following sections, we discuss the role of various GM modulatory therapies in human trials.[56]

Antibiotic Therapies in Liver Disease

Antibiotics are primarily used for their antibacterial efficacy, but they also alter the composition of the commensal gut microbial population. Bacterial infection is more likely in those with cirrhosis, particularly decompensated cirrhosis, which may promote further hepatic decompensation, including liver failure. Due to their negative effects and the growth of antibiotic resistance, conventional antibiotics may not be useful in modulating microbiota in the long run. Studies on norfloxacin in decompensated cirrhosis, vancomycin in PSC, and amoxycillin in severe AH have yielded mixed results on short-term GM modulation without long-term clinical benefits. In a randomized controlled trial (RCT) of patients with advanced cirrhosis without recent fluoroquinolone therapy, researchers found that norfloxacin did not reduce 6-month mortality, but increased survival in those with low ascites fluid protein concentration and reduced the incidence of gram-negative bacterial infection.[57] A recent abstract study (Antibiocor Trial) found that combining amoxicillin/clavulanate antibiotics with prednisolone did not improve survival in patients with severe AH.[58] Another study found that gut-selective, broad-spectrum antibiotics did not affect bacterial translocation or hepatic and systemic inflammation in AH.[59]

Rifaximin is the most studied antibiotic as a GM modulator in liver disease and has modulatory effects on the intestinal bacterial population and local and systemic inflammatory biomarkers. It reduces the hepatic venous pressure gradient; improves the pathogenic abundance of Veillonellaceae; reduces liver disease severity scores and endotoxemia via intestinal decontamination; decreases secondary to primary bile acid ratios; and improves cognition, systemic inflammation, liver enzymes, insulin resistance markers, and quality of life in patients with HE in the nonalcoholic fatty liver disease (NAFLD), HBV, and ALD groups. However, large, high-quality studies and validations from early observations on the actuarial, real-world efficacy of rifaximin as a direct modulator of GM and, thus, clinical outcomes are required.[19,43,60]

Probiotics, Prebiotics, and Synbiotics

Most studies on the clinical benefits of probiotics, prebiotics, or synbiotics as GM modulators used small animal models. Clinical studies in humans have yet to be fully validated and replicated in the context of probiotics or prebiotics, even though some studies have shown the benefits of using probiotics in certain liver disease conditions. Thus, most therapeutic applications of prebiotics, probiotics, and synbiotics in liver disease remain experimental. Probiotics, prebiotics, and synbiotics have improved investigational variables in NAFLD, NASH, ALD, HBV, and PSC. High-dose probiotics containing mostly *Lactobacillus salivarius*, *Lactobacillus lactis*, *Lactobacillus plantarum*, *L. reuteri*, *Bifidobacterium* species, *Propionibacterium*, and multistrain products with prebiotics such as inulin, guar gum, and pectin have shown nonsustained improvement in liver tests, inflammatory biomarkers, and insulin resistance components without any appreciable benefits on symptoms and signs, liver fibrosis, liver disease severity, or long-term clinical outcomes. Probiotic therapy in decompensated cirrhosis patients has shown some benefits in improving cognition and reducing HE episodes when used in conjunction with standard care. These advantages were linked to increased therapeutic gut bacteria strains and a decrease in potentially pathogenic species such as *Enterococcus* and Enterobacteriaceae.[56] According to recent meta-analyses of human studies, probiotics/prebiotics/synbiotics may improve energy metabolism, inflammation, and liver function biomarkers as well as liver histology in the MAFLD population. These effects, however, require confirmation through additional research.[61,62] Similarly, the

high-quality meta-analysis revealed that probiotic-based microbial treatments for ALD could modestly reverse dysbiosis, affecting lipid metabolism, relieving inflammatory response, and inhibiting oxidative stress to improve liver function. However, these were not clinically evident in severity scores or survival outcomes.[63]

Despite the dearth of human clinical trials, numerous animal model studies have observed the benefit of probiotics and postbiotics (microbial components or soluble biologically active compounds often created by probiotics by employing prebiotics) in reducing acetaminophen-induced liver impairment. *Enterococcus lactis*, *S. salivarius*, *Bacillus*, *Lactobacillus ingluviei*, *Lactobacillus acidophilus*, *Lactobacillus rhamnosus GG*, and *A. muciniphila* were all shown to modulate the immunological milieu in rat and mouse intestines resulting in a reduction in acetaminophen-induced liver injury.[64] According to an uncontrolled clinical study, long-term administration of the probiotic *E. faecalis* strain FK-23 to patients with chronic viral hepatitis C reduced liver enzymes while having no appreciable effect on viral load, blood total protein, urea, and hemoglobin levels or platelet count. A relatively small RCT of patients with PSC and inflammatory bowel disorders showed that probiotics had no discernible effect on pruritus, tiredness, serum bilirubin levels, liver enzymes, prothrombin, albumin, or bile salts.[65] A recent study compared the clinical outcomes and gut microbiome changes associated with high-dose probiotic infusion (HDPI) therapy to corticosteroids and FMT in patients with severe AH. The authors discovered that HDPI did not improve clinical outcomes better than corticosteroids. It was linked to sparse quantitative and qualitative changes in bacterial taxa such as *Bilophila* and *Roseburia* and the persistence of potentially pathogenic bacterial communities and their interactions.[66] The clinically significant benefits of probiotic/prebiotic or postbiotic therapy in chronic viral hepatitis, AIH, syndrome of ACLF and ALF, and chronic cholestatic liver disease require more well-designed prospective trials.

Fecal Microbiota Transplantation

FMT is an infusion of freshly prepared or thawed frozen suspension of stool from a rigorously screened healthy individual to an individual with a disease to treat the specific disease or complication by ameliorating gut dysbiosis[67] (►**Fig. 4**).

Chronic Hepatitis B Virus Infection

A study evaluated the efficacy of FMT in hepatitis B e-antigen (HBeAg)–positive patients receiving entecavir and tenofovir treatment. Despite receiving long-term antiviral therapy, many patients with persistent HBeAg responded favorably to FMT treatment resulting in HBeAg clearance. In patients with chronic hepatitis B infection, FMT may be an effective adjuvant therapeutic option for regulating intestinal microbiota, according to the findings of this study.[68] In an Indian study, the effect of FMT on the levels of hepatitis B surface antigen, HBeAg, and viral DNA was investigated. In patients with HBeAg-positive chronic hepatitis B infection, a nonrandomized pilot trial found that FMT was safe and possibly effective regarding viral suppression and HBeAg clearance.[69] Furthermore, there is an unmet need for carefully controlled research on the effects of FMT at different stages of HBV infection, particularly its impact on carcinogenesis.

Cirrhosis and Its Complications

FMT from prespecified healthy stool donors reduced HE in cirrhotic patients. The researchers discovered that Proteobacteria expansion was linked to a decrease in beneficial taxa and microbial diversity in antibiotic-treated patients, which was alleviated by FMT.[70] Compared with conventional therapy, a 1-year follow-up of this trial revealed that FMT effectively prevented recurrent HE and hospitalization, and was safe in the long term. Burkholderiaceae expanded post-FMT, while Acidaminoccocaceae declined.[71] In a phase 1 study, oral capsule–based FMT was linked to increased Ruminococcaceae and Bifidobacteriaceae and decreased Streptococcaceae and Veillonellaceae, resulting in an improvement in duodenal mucosal diversity. According to this study, FMT helped cirrhosis patients with HE and dysbiosis, increased antimicrobial peptide production in the gut, reduced endotoxemia, and improved cognitive scores. Beneficial taxa associated with improved cognition and reduced systemic inflammation, such as Ruminococcaceae, Verrucomicrobiaceae, and Lachnospiraceae, thrived in

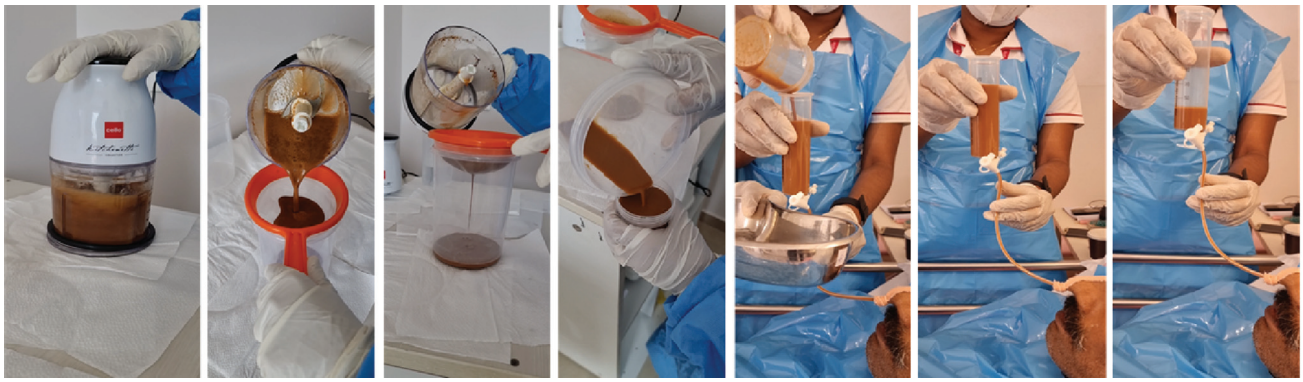

Fig. 4 The procedure of fresh fecal transplant via the nasoduodenal tube. The basic steps include homogenization, filtering, transfer, and instillation.

the guts of cirrhotic patients following capsule FMT.[72] According to an Indian research, one session of colonoscopic FMT for recurrent HE resulted in a long-term beneficial clinical response in 60% of patients after 20 weeks, which was also associated with a reduction in the severity of liver disease.[73]

Another study discovered that antibiotic use altered the composition of the GM and its beneficial metabolic functions in cirrhosis, which were restored via FMT. After FMT, beneficial taxa such as Lachnospiraceae and Ruminococcaceae expanded significantly. Before and during capsular or rectal FMT, researchers assessed the prevalence of the antibiotic resistance gene in cirrhotic patients. Moreover, the expression of antibiotic resistance genes for vancomycin, β-lactamase, and rifamycin was lower in FMT-exposed patients than those receiving a placebo.[74] Bloom et al discovered that patients who responded to capsule FMT had higher levels of *Bifidobacterium* (*B. angulatum* and *B. adolescentis*, both SCFA producers) and other known beneficial taxa throughout the study. The FMT donor whose recipients had the worst cognitive outcomes had the lowest fecal SCFA levels. The authors concluded that FMT capsules improved cognition in HE; the effect varied depending on donor and recipient factors.[75]

MAFLD and NASH

In patients with MAFLD, FMT studies are limited in the clinical setting. According to one study, a single infusion did not affect hepatic steatosis. In another study, 3 days of stool infusions resulted in a slight but significant reduction in the severity of steatosis. There were no significant changes in insulin resistance indices or magnetic resonance imaging–based liver fat estimation in patients who received allogeneic or autologous FMT. Six weeks after allogenic FMT, patients with elevated small intestinal permeability at baseline had a significant reduction. An RCT found that allogenic FMT using lean vegan donors in people with hepatic steatosis improved intestinal microbiota composition, linked to improved plasma metabolites and steatohepatitis markers.[76–78]

Alcohol-Related Liver Disease

In an open-label trial, patients with severe AH who received 1 week of FMT through a nasojejunal tube inserted under fluoroscopic guidance had greater transplant-free survival than matched historical controls. One year after FMT, the gut microbiome of recipients were enriched in phylum Firmicutes, similar to donors at baseline. After treatment, there was a decrease in harmful Proteobacteria and an increase in beneficial, SCFA-producing Actinobacteria. At 6-month and 1-year follow-up after FMT, relative abundances of nonpathogenic species such as *Megasphaera elsdenii*, *Bifidobacterium longum*, and *Enterococcus villorum* increased, while relative abundances of *K. pneumonia* decreased.[79] The clinical outcomes of patients with severe AH receiving FMT, pentoxifylline, corticosteroid, or nutritional treatment were compared in a second open-label trial conducted by the same research team with a 3-month follow-up period. FMT was the most effective intervention for increasing survival and was associated with the beneficial modification of bacterial populations and their functional metabolism. After 1 week and 30 days following FMT, harmful taxa such as *Bilophila*, *Enterobacter*, and *Klebsiella* decreased, while beneficial species such as *Bacteroides*, *Parabacteroides*, and *Porphyromonas* expanded. In contrast to LPS signaling pathways, peroxisome proliferator-activated receptor signaling pathways were markedly upregulated in FMT patients.[80] In the longest follow-up study on FMT in severe AH, stool transplant was associated with significantly fewer ascites, infections, encephalopathy, and alcohol relapse (with a trend toward higher survival rates) than standard care, which was also associated with beneficial GM modulation.[81] Severe AH-related ACLF is a lethal condition frequently associated with high mortality and corticosteroid nonresponse. Higher grades of ACLF (classes 2 and 3) have a 3-month survival rate of only 36.7%. A pilot study on FMT in patients with AH-related ACLF found an overall survival rate of 66% after 548 days of follow-up. After 548 days, 58.3% of ACLF patients with higher grades survived, demonstrating the benefits of FMT in this critically ill population.[82] Concurrent with previous findings, an Indian study on AH-related ACLF found that FMT was safe, improved short- and medium-term survival, and reduced clinical severity scores in patients.[83] An RCT of FMT versus prednisolone demonstrated safety, improved 90-day survival, and decreased infections by modulating microbial communities favorably.[84] In a different study, patients with severe AH were treated with FMT or pentoxifylline, and clinical outcomes and GM characteristics were compared. These results showed that healthy-donor FMT improved survival rates and decreased liver-related complications compared with pentoxifylline, which was linked to favorable modulation of intestinal bacterial communities. Bifidobacterium in the FMT group and pathogenic Aerococcaceae in the pentoxifylline group increased at 6-month follow-up. Beneficial taxa (*Bifidobacterium*) were found to be a key influencer in those undergoing FMT at 6 months, according to network analysis.[85] Finally, preclinical trials have shown that engineered microbes, including bacteriophages targeted at specific bacteria, play an important role in the etiology and progression of severe AH.[86] Phage therapy, synthetic microbial therapy, and synthetic precision medicine–based multimicrobial therapy (synthetic stool) could be valuable additions to the microbial therapeutic arsenal developed to treat various liver diseases. ►**Table 3** summarizes FMT in liver disease in the context of human studies.

Primary Sclerosing Cholangitis

In the first case report on the impact of FMT on a patient with PSC, the patient received weekly FMT for 4 weeks, resulting in a decrease in bile acids and alkaline phosphatase. Jaundice disappeared for up to a year after receiving FMT. A significant long-term decrease in the number of harmful Proteobacteria and an increase in the beneficial Firmicutes phyla have been observed.[87] A pilot clinical study in PSC patients who underwent FMT and had a

Table 3 Human studies on fecal microbiota transplantation in cirrhosis and severe liver disease and associated clinical events

Study (year)	Type	Patients	Salient features
Fecal microbiota transplantation			
Philips CA (2017)	Pilot study Open-label Fresh FMT Nasoduodenal tube 100 mL once daily for 7 d	• $N = 8$ • Historical controls Steroid-ineligible patients • 12-mo follow-up	• FMT significantly increased patient survival • Nonpathogenic *Enterococcus villorum*, *Bifidobacterium longum*, and *Megasphaera elsdenii* increased post-FMT at 6–12 mo
Bajaj JS (2017)	Randomized Double-blind	• $N = 10$ • Recurrent encephalopathy patients • Before receiving a single FMT enema from donor stool enriched in Lachnospiraceae and Ruminococcaceae, patients received a 5-d course of a broad-spectrum antibiotic • 3 mo of monitoring	• Antibiotics reduced diversity, autochthonous taxa, and increased Proteobacteria • Lactobacillaceae, Bifidobacteriaceae, Lachnospiraceae, and Ruminococcaceae increased post-FMT
Philips CA (2018)	Retrospective Open-label	• FMT, $N = 16$ • Steroids, $N = 8$ • Pentoxifylline, $N = 10$ • Nutritional therapy, $N = 17$ • Alcohol-associated hepatitis • Survival at 90 d	• The FMT group had the highest survival rate • *Porphyromonas* and Parabacteroides predominant at baseline among healthy controls. • *Veillonella*, *Dialister*, *Lentisphaera*, and *Victivallis* dominated in patients prior to therapy • 1 mo after FMT, *Roseburia* and *Micrococcus* rose • After FMT, lipopolysaccharide synthetic pathways were downregulated
Bajaj JS (2019)	Randomized Placebo-controlled Phase 1	• $N = 20$ (10 in each) • Recurrent encephalopathy • FMT in capsule form • Stool from single donor enriched in Ruminococcaceae and Lachnospiraceae • 90-d follow-up	• Increased diversity of mucosal gut microbiota • Higher Bifidobacteriaceae and Ruminococcaceae • Lower Veillonellaceae and Streptococcaceae • Veillonellaceae levels in sigmoid and stool decreased • Post-FMT elevation of duodenal mucosal defensin α and E-cadherin • Serum lipopolysaccharide-binding protein and IL-6 reduced after FMT
Bajaj JS (2020)	Extended analysis of a prior study	• $N = 7$ • Recurrent encephalopathy • Lachnospiraceae- and Ruminococcaceae-enriched single-session FMT enema • Lactulose, rifaximin, and proton-pump inhibitors were administered to all patients • More than 1-y follow-up	After FMT • Burkholderiaceae expanded • Declining Acidaminoccocaceae • Across groups, Lachnospiraceae and Ruminococcaceae remained identical
Bajaj JS (2020)	Placebo-controlled randomized trial	• $N = 20$ in each arm • Alcohol use disorder cirrhosis patients • Donor stool enriched in Lachnospiraceae and Ruminococcaceae administered as single session FMT enema • 180-d follow-up	• By day 15, 90% receiving FMT saw considerable craving reduction compared with 30% on placebo After FMT: • Reduced ethyl glucuronide/creatinine levels in the urine • Decreased blood IL-6 and lipopolysaccharide-binding protein; improved cognition and psychosocial quality of life; increased butyrate/isobutyrate relative to baseline in FMT but not placebo • FMT but not placebo resulted in more Ruminococcaceae • Among severe adverse events, AUD-related adverse events were more common in the placebo group

(*Continued*)

Table 3 (*Continued*)

Study (year)	Type	Patients	Salient features
Bajaj JS (2020)	Follow-up analyses of two randomized controlled trials in depth	• Decompensated cirrhosis • 20 participants in each trial	• In comparison to baseline, β-lactamase expression dropped after FMT • Vancomycin, β-lactamase, and rifamycin antibiotic resistance gene expression were less prevalent after FMT • Vancomycin and β-lactamase antibiotic resistance gene expression increased in the antibiotics + enema experiment, and quinolone resistance increased at day 15 compared with baseline • In decompensated cirrhosis, antibiotic resistance gene expression abundance was significantly lower after FMT compared with pre-FMT baseline and non-FMT groups
Philips CA (2021)	Retrospective Open-label Comparison with corticosteroids	• FMT, $N = 35$ • Corticosteroids, $N = 26$ • 3-y follow-up	• The steroid group experienced much greater incidence of ascites, encephalopathy, infections, and severe hospitalization than the FMT group • Alcohol relapse was less common (53.8 vs 28.6%), and the FMT treated had a longer period until relapse than those on steroids • The FMT group had a greater 3-y survival rate (65.7 vs 38.5%) than the steroid group • In the FMT group, there was a significant rise in Bifidobacterium and a decline in Acinetobacter • *Porphyromonas* levels were noticeably greater and Bifidobacterium levels were lower in the those exposed to steroids compared with FMT beyond 48 wk
Sharma A (2022)	Open-label trial Comparison with corticosteroids	• FMT, $N = 13$ • Corticosteroids, $N = 20$ • Alcohol-associated acute-on-chronic liver failure • Single session fresh FMT of 100 mL via nasoduodenal route	• Survival at 28 and 90 d was significantly better in the FMT arm (100 vs 60%) • Hepatic encephalopathy resolved in 100 vs 57.14% (FMT vs steroids) • Ascites resolved in 100 (FMT) vs 40% survivors (in the steroid group) • FMT was safe, improves short-term and medium-term survival, and improves clinical severity scores
Pande A (2022)	Open-label Randomized study Comparison with corticosteroids	• $N = 60$ in each arm • Severe alcohol-associated hepatitis • Fresh FMT via nasoduodenal route, 100 mL daily for 1 wk • 3-mo follow-up	• In terms of survival, FMT was superior to steroid therapy • 28 d into FMT, 23 new taxa had emerged • Anaerobes (*Parcubacteria*, *Weissella*, and Leuconostocaceae) and pathogenic taxa (*Campylobacter*) decreased from baseline levels, whereas Alphaproteobacteria and *Thaumarchaeota* increased • By more positively modifying microbial populations than prednisolone, FMT decreased infections, increased 90-day survival, and was safer
Philips CA (2022)	Open-label trial	• Pentoxifylline, $N = 20$ • FMT, $N = 47$ • Severe alcohol-associated hepatitis • 180-d follow-up	• Those who underwent FMT had a greater 6-mo survival rate than patients who received pentoxifylline (83 vs 56%) • Patients who received pentoxifylline had significantly higher rates of clinically significant ascites (56 vs 25.5%), hepatic encephalopathy (40 vs 10.6%), and serious infections (52 vs 14.9%) • Bifidobacterium in the FMT group and pathogenic Aerococcaceae in the pentoxifylline group stood out after 6 mo • Compared with pentoxifylline, FMT from healthy donors increased survival rates and decreased direct liver-related events

Abbreviations: AUD, alcohol use disorder; FMT, fecal microbiota transplantation; IL, interleukin.

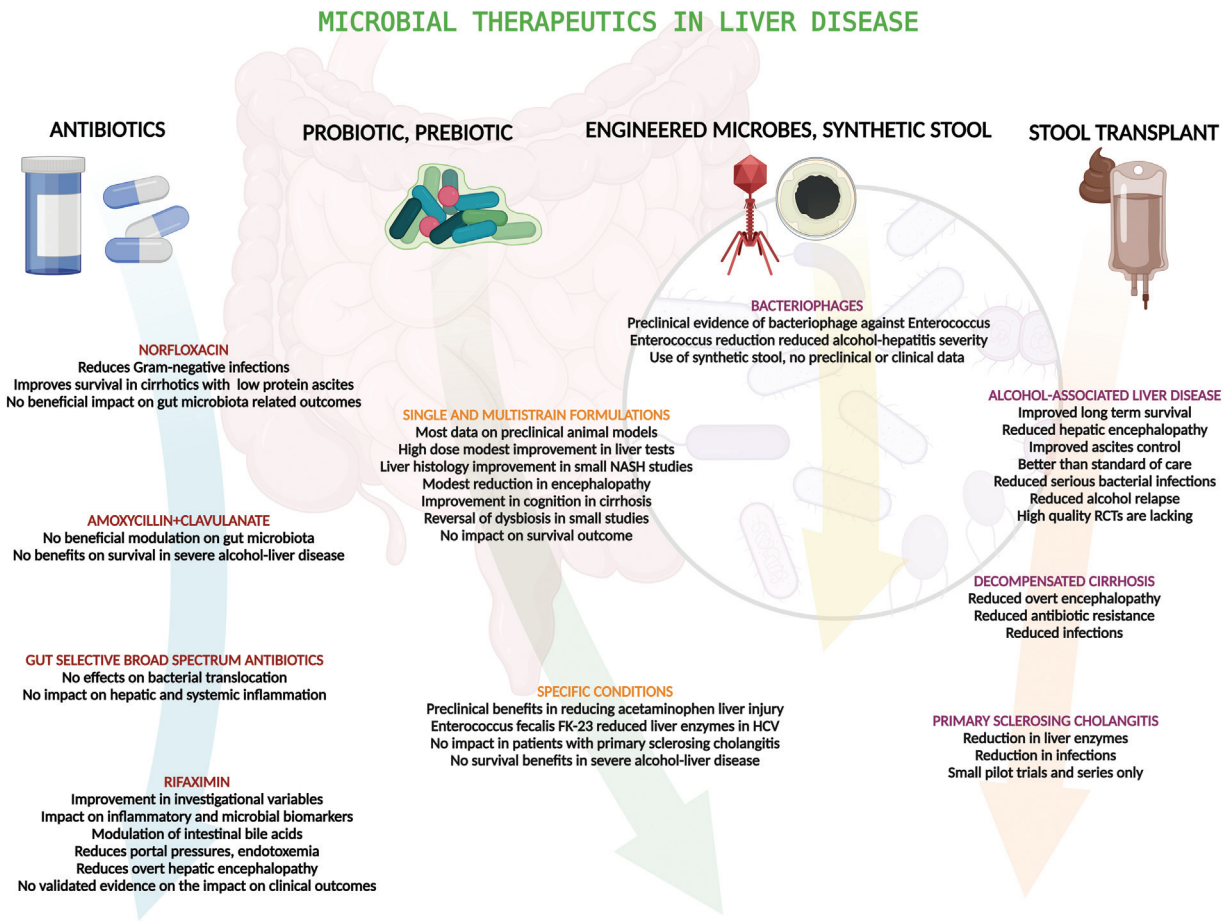

Fig. 5 A summary of various microbes-based therapeutic approaches in liver disease.

mean alkaline phosphatase level of 489 U/L revealed that 30% of patients had a 50% reduction in alkaline phosphatase levels. The efficacy and safety of FMT in PSC were first reported in this prospective trial.[88] There is an unmet need for large, well-controlled trials to examine clinical outcomes associated with FMT in PSC patients with and without inflammatory bowel disease, such as disease progression (fibrosis) and clinical consequences like biliary infections.[54]

Conclusion

GM significantly influences the development of acute and chronic liver disorders and clinical consequences linked to progressive liver failure and portal hypertension. Dysbiotic gut flora, influenced by intestinal permeability, is primarily linked to the onset and progression of various liver diseases. The release of intestinal inflammatory factors affects the host at the local tissue and systemic organ levels and aids liver disease development and clinical progression. Developing microbial therapies that can successfully reduce disease severity and delay the progression of cirrhosis and its complications has significant clinical implications. Probiotics, prebiotics, and synbiotics, various antibiotics (absorbable and nonabsorbable), and stool transplant–based modulation have mostly been tested in human studies with a consistent demonstration of clinical benefits associated with microbial manipulation (►**Fig. 5**). However, more well-designed RCTs involving patients with liver disease are needed to assess the efficacy and safety of microbiota-centered treatments.

Author Contributions

C.A.P.: conceptualization, writing - original draft, writing - review and editing. P.A.: supervision, writing - review and editing.

Conflict of Interest

None declared.

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