The gut microbiota and the liver. Pathophysiological and clinical implications

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Introduction

The term microbiota is used to describe the complete population of microorganisms that populate a certain location, such as the gut, and is preferred to the term flora as the former incorporates not just bacteria but also archaea, viruses, and other microorganisms, such as protozoa. Though the potential role of the microbiota (through such concepts as "the putrefactive principle associated with faeces" and "intestinal toxins") in the pathogenesis of systemic disorders has been recognized since antiquity, a firm scientific basis for a role for the gut microbiome in liver disease did not emerge until the middle of the last century with the recognition of the relationship between hepatic coma and the absorption of nitrogenous substances from the intestine [1]. This was followed by the description of abundant coliforms in the small intestine of cirrhotics [2] and the role of bacteria was clinched by trials demonstrating that antibiotics led to clinical improvement in hepatic encephalopathy (HE) [3]. Subsequently, these same gut-derived bacteria were implicated in another complication of chronic liver disease and portal hypertension, spontaneous bacterial peritonitis. Most recently, more credence has been given to a suggestion that has lingered in the background for decades, namely, that the gut microbiota might play a role in the pathogenesis or progression of certain liver diseases, including alcoholic liver disease [4], non-alcoholic fatty liver disease (NAFLD) and non-alcoholic steato-hepatitis (NASH) [5], total parenteral nutrition (TPN)/intestinal failure-related liver disease (IFALD) [6], and primary sclerosing cholangitis (PSC) [7], either through the direct effects of bacteria or their products, via inflammatory mediators such as tumor necrosis factor α (TNF), whose release had been triggered by constituents of the microbiota, or, as in the case of primary sclerosing cholangitis (PSC), through cross-reactivity between microbial antigens and human tissue components (e.g., atypical anti-nuclear cytoplasmic antibodies (p-ANCA), in PSC, recognize both tubulin beta isoform 5 in human neutrophils, and the bacterial cell division protein FtsZ) [8].

Indeed, inflammatory mediators have also been implicated in the development and maintenance of the hyperdynamic circulation that is a feature of portal hypertension [9], in impairing liver function and contributing to haemostatic failure [10]. It is in these contexts that modulation of the microbiota has emerged as a potential therapeutic strategy in the management of liver disease.

The microbiota in the pathogenesis of liver disease and its complications

The human intestinal microbiota consists of trillions of microorganisms including 150–200 prevalent and ∼1000 less common bacterial species, harbouring over 100-fold more genes than those present in the human genome [11–13]. This microbiota consists predominantly of bacteria, but also contains archaea, protozoa, and viruses; all have co-evolved with the human host. The microbiota performs vital functions essential to health maintenance, including food processing, digestion of complex indigestible polysaccharides and synthesis of vitamins [14,15]. Furthermore, it secretes a range of bioactive metabolites with diverse functions, ranging from inhibition of pathogens, metabolism of toxic compounds to modulation of host metabolism [15]. A perturbed microbiota has been implicated in an ever-increasing list of disorders in humans, from necrotizing enterocolitis (NEC) in infants, to obesity, diabetes, metabolic syndrome, irritable bowel syndrome (IBS), and inflammatory bowel disease (IBD) in adults [16–21]. However, little is known about the physiological impact the microbiota has on host health, an area which has recently been described as "one of the hottest areas in medicine" [22].

Table 1 lists a number of mechanisms that might underpin the role of the microbiota in the pathogenesis of liver disease and/or its complications.

Small intestinal bacterial overgrowth (SIBO)

Among the various potential contributions of the microbiota to liver disease, small intestinal bacterial overgrowth (SIBO) has been one of the most extensively studied. Indeed, an altered gut

Key Points

- The microbiota performs vital functions including food processing, digestion of complex indigestible polysaccharides and synthesis of vitamins

- The advent of molecular techniques now permits the complete delineation of the constituents of the microbiota in health and disease

- Changes in the microbiota have been identified in relation to a number of liver diseases and implicated in the pathophysiology of NAFLD/NASH, alcoholic liver disease, and IFALD, in particular

- Small intestinal bacterial overgrowth has been associated, in cirrhosis, with bacteraemia, SBP, and HE

- Modulation of the microbiota, by antibiotics, is an established strategy in the management and prevention of a number of complications of chronic liver disease

- While probiotics hold promise in the management of liver disease, and their potential is supported by a considerable volume of laboratory work, high-quality clinical evidence is scanty

pathophysiology of barrier dysfunction is unclear, roles for metabolites of alcohol and/or pro-inflammatory cytokines have been postulated [4,5,7,30,31]. Impaired antimicrobial defence mechanisms may further contribute to the development of bacterial translocation in portal hypertension and cirrhosis [30]. Taken together, these observations have led to the development of a schema which links intestinal motility, stasis, increased intestinal permeability and translocation of bacteria and endotoxin to the development of many of the complications of liver disease, as well as in the progression of liver disease, *per se* (Fig. 1).

A more fundamental role for SIBO has been proposed in both alcoholic liver disease (through the generation of acetaldehyde) [31] and NAFLD (by promoting both steatosis and inflammation) [5]. The potential of microbes of enteric origin to induce a progressive and even fatal steato-hepatitis had been recognized some years ago in relation to the liver injury that complicated jejuno-ileal bypass operations for morbid obesity; indeed, that procedure has provided a valuable experimental model for exploring the impact of the microbiota in liver disease.
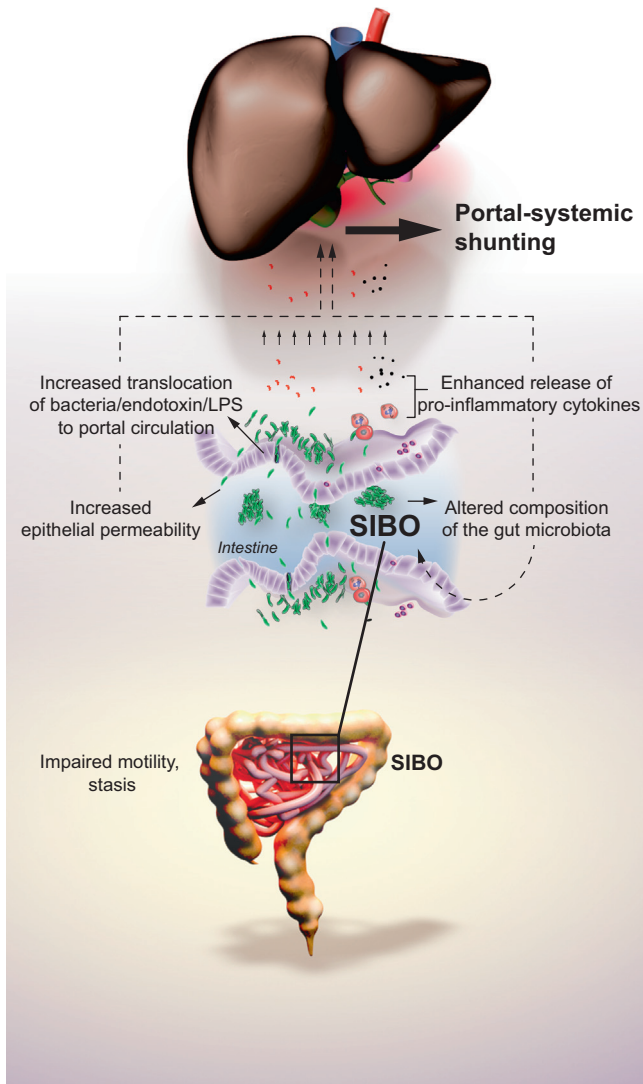
Quantitative or qualitative changes in the microbiota

The advent of molecular techniques based on sequence divergences of the small subunit ribosomal ribonucleic acid (16S rRNA) of bacteria [32] now permits the complete delineation of the constituents of the microbiota in health, thereby, revealing the composition of the human gut microbiota from early infancy [33] through to old age [34,35]. Following birth, the human intestine is rapidly colonized and factors known to influence colonization include gestational age, mode of delivery (vaginal birth *vs.* assisted delivery), diet (breast milk *vs.* formula), level of sanitation, and exposure to antibiotics [15,36]. The intestinal microbiota of newborn infants is characterized by low diversity and a relative dominance of the phyla *Proteobacteria* and *Actinobacteria*; thereafter, the microbiota becomes more diverse with the emergence of the dominance of *Firmicutes* and *Bacteroidetes*, which characterises the adult microbiota [12,14,37]. By the end of the first year of life, infants possess a microbial profile distinct for each infant; by 2.5 years of age, the microbiota fully resembles that of an adult in terms of composition [33,38]. More recently, three different 'enterotypes' have been described in the adult human microbiome [39] These distinct 'enterotypes' are dominated by *Prevotella*, *Ruminococcus*, and *Bacteroides*, respectively,

microbiota was first noted by Hoefert in chronic liver disease over 80 years ago [23]; since then SIBO has been documented to be common in liver disease, to correlate with its severity and to be linked to minimal and overt encephalopathy and an increased risk for SBP through translocation across the gut wall [24–28]. Moreover, factors predisposing to SIBO such as altered small bowel motility, increased intestinal permeability, and delayed gut transit have been reported in patients with liver cirrhosis [29]. Alterations in gastrointestinal motility in cirrhosis have been variably ascribed to the effects of autonomic dysfunction, altered levels of circulating neuropeptides and the effects of inflammatory mediators on gut muscle and nerve [9,29]. With regard to intestinal permeability, changes in intestinal tight junctional proteins have been described in cirrhosis and, though the precise

Table 1. The microbiota in the pathogenesis of liver disease and its complications. Possible factors.

Microbial factor	Examples of mediator(s)	Clinical implications
Small intestinal bacterial overgrowth	Multiple	NAFLD/NASH IFALD
Alterations in the composition of the microbiota	Multiple	Obesity
Translocation of bacteria or bacterial components	Lipopolysaccharide, endotoxin	SBP Other infectious complications of liver disease Portal hypertension
Direct effects of products of gut bacterial metabolism	Acetaldehyde, TMA, TMAO	Alcoholic liver disease NAFLD/NASH
Immune response to a normal or abnormal microbiota	Cross-reactivity between bacterial antigens and host tissue components	PSC
Effects of cytokines released in response to the microbiota	TNFα	NAFLD/NASH Portal hypertension

NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis; IFALD, intestinal failure-associated liver disease; SBP, spontaneous bacterial peritonitis; TMA, trimethylamine; TMAO, trimethylamine oxide; PSC, primary sclerosing cholangitis; TNFα, tumor necrosis factor alpha.

Fig. 1. A schema to explain potential roles of the small intestinal bacterial overgrowth (SIBO) in liver disease and its complications. Impaired motility promotes stasis; quantitative and qualitative changes in the small intestinal microbiota ensue leading to the development of small intestinal bacterial overgrowth (SIBO). Alterations in the integrity of the intestinal barrier result in enhanced permeability and facilitate the translocation of bacteria or their products into the portal circulation. Changes in the enteric microbiota also lead to the enhanced release of pro-inflammatory cytokines which enhance translocation and exert a variety of effects on liver structure and function. If portal-systemic shunting is present, access for the products of translocation and cytokines to the systemic circulation is enhanced.

and their appearance appears to be independent of sex, age, nationality and body mass index (BMI). Once the microbiota has reached maturity, it is thought to remain stable until old age when changes are seen, possibly related to alterations in digestive physiology and diet [13,40,41]. Indeed, Claesson *et al.* were able to identify clear correlations, in the elderly, not only between the composition of the gut microbiota and diet, but also with health status [35].

These molecular approaches have, to some extent, been directed at the investigation of the microbiota in liver disease. Chen *et al.*, for example, studied 36 patients with cirrhosis and 24 healthy controls using 454 pyrosequencing of the 16S ribosomal

RNA variable region 3 (V3) followed by real-time quantitative polymerase chain reaction (qPCR) and found that, in cirrhosis, *Bacteroidetes* were reduced, *Proteobacteria* and *Fusobacteria* increased, *Enterobacteriaceae*, *Veillonellaceae*, *Streptococcaceae* increased, and *Lachnospiraceae* reduced [42]. Some of these changes correlated with clinical parameters such as the Child-Turcotte-Pugh score. Several of these findings have been confirmed by Bajaj *et al.* who went on to show that *Veillonellaceae* were more abundant among cirrhotics with hepatic encephalopathy (HE) than those without [43]. As an extension of the evolving concept of the microbiota–gut–brain-axis, which includes the suggestion that the microbiota can influence cognition and behaviour, these authors also described an association between certain bacterial families, cognition and inflammation in HE. The finding of an increased abundance of members of the family *Enterobacteriaceae* in cirrhosis by these and other authors [44] is of interest given that this family includes such important Gram-negative pathogens as *Salmonella*, *Shigella*, *Yersinia pestis*, *Klebsiella* and, especially, *Escherichia coli*, a common isolate in SBP and bacteremia in chronic liver disease. Others have documented, not only a decrease in total bifidobacterial counts, but also further variations in specific *Bifidobacterium* species (e.g., *Bifidobacterium dentium* increased; *Bifidobacterium longum* decreased) in chronic liver disease [45].

Interactions with diet and nutrients

Several mechanisms have been identified relevant to the involvement of the microbiota in the pathogenesis of NAFLD/ NASH. In particular, a role for the microbiota and its interaction with diet in the pathogenesis of obesity *per se* has been extensively investigated [46,47], and pertinent findings include the ability of Gram-negative anaerobes, such as *Bacteriodes thetaiotamicron*, to cleave most glycosidic linkages, degrade plant polysaccharides and, thereby, supply the host with 10–15% of its calorific requirement [11,17,46,47]. The microbiota of obese individuals, as well as the caecal microbiota of *ob/ob* mice, are more efficient at the extraction of energy from the diet and in the production of short chain fatty acids (SCFAs) [11,48]. Furthermore, the microbiota has been shown to stimulate hepatic triglyceride production through suppression of the lipoprotein lipase (LPL) inhibitor, fasting-induced adipose factor (Fiaf; also known as angiopoietin-like 4), thereby leading to continued expression of LPL, a key regulator of fatty acid release from triglycerides in the liver [49]. The gut microbiota can also modulate systemic lipid metabolism through modification of bile acid metabolic patterns, impacting directly on the emulsification and absorption properties of bile acids and thus, indirectly, on the storage of fatty acids in the liver. The microbiota has also been implicated in the development of insulin resistance [49], a fundamental abnormality in the metabolic syndrome, by affecting energy balance, glucose metabolism, and the low-grade inflammatory state that has been associated with obesity and related metabolic disorders. Its role in choline metabolism [50–52], as well as in activation of pro-inflammatory cytokines (e.g., TNFα), appears relevant to the development of NAFLD and progression to NASH. Most recently, studies in experimental models have shown that defective/deficient inflammasome sensing and related dysbiosis result in an abnormal accumulation of bacterial products in the portal circulation and promote progression of NAFLD/NASH [53].

Interactions with alcohol

Alcohol-related liver injury may also involve the active participation of the microbiota through a number of mechanisms [31]. Firstly, alcohol promotes the growth of Gram-negative bacteria in the gut with the consequent production of endotoxin. Secondly, Gram-negative bacteria metabolize alcohol to acetaldehyde which, in turn, increases intestinal permeability. Other effects of alcohol conspire to increase permeability, promote transfer of endotoxin across the intestinal epithelium and impair the host immune response [31]. Protein adducts formed from metabolites of alcohol have also been shown to combine with LPS to induce liver injury [54].

Therapeutic impact of modulating the microbiota

Antibiotics

Infectious complications. Reflecting the involvement of the microbiota in the genesis of the infectious complications of liver disease [55], the fundamental role of antibiotic therapy directed at Gram-negative enteric bacteria, in the management and prevention of infectious complications of liver disease and portal hypertension and chronic liver disease, such as SBP and bacteraemia, is well established [56]. Indeed, antibiotics have also been advocated to both prevent infectious complications and reduce re-bleeding rates among patients with variceal haemorrhage [10,56,57]. Based on the aforementioned role of endotoxemia resulting from bacterial translocation in the pathogenesis of the hyperdynamic circulation that characterizes portal hypertension (through the induction of nitric oxide [NO] synthase), selective intestinal decontamination has also been proposed as a strategy in the management of the circulatory complications of cirrhosis [58].

Hepatic encephalopathy

The recent studies, indicating the efficacy of the poorly absorbed antibiotic with activity against enteric organisms, rifaximin, in overt [59] and sub-clinical [60] HE, have bolstered the importance of the role of these organisms in the pathogenesis of this common complication of liver disease; the precise nature of the changes in the composition and/or metabolic activity of the microbiota that generate these benefits remains to be determined.

Intestinal failure-associated liver disease (IFALD)

The term intestinal failure-associated liver disease has replaced that of total parenteral nutrition (TPN)-associated liver disease to describe alterations in liver morphology and function that occur among subjects with intestinal failure who may or may not also be on TPN. The etiology of intestinal failure-associated liver disease (IFALD) is multifactorial, with primary contributors including prematurity (in infants), sepsis, nutritional deficiencies or hepatotoxic effects of TPN infusates, and lack of enterally stimulated bile flow [61]. Though SIBO may be an important feature in intestinal failure of various aetiologies and has been implicated in the pathogenesis of IFALD, there is currently insufficient evidence to support the use of antibiotics, such as metronidazole, in the prevention of IFALD [62].

Probiotics and prebiotics

Experimental

NAFLD: In a pioneering study in the *ob/ob* mouse (an animal model of NAFLD), Li *et al.* compared the probiotic cocktail VSL#3 to an anti-TNFα antibody. Though, in comparison to the anti-TNFα antibody, VSL#3 had less effect on steatosis, its impact on inflammation and liver injury was similar, yet did not appear to be exerted through a reduction in hepatic expression of TNFα [63]. In a model that is more relevant to humans, diet-induced obesity, Ma *et al.* showed that the same probiotic preparation could prevent the development of steatosis through a natural killer T-cell (NKT)-dependent mechanism [64]; in contrast, other workers found that VSL#3, while attenuating fibrosis, had no effect on either steatosis or steatohepatitis in NASH induced by a methionine-choline-deficient diet [65]. Based on their studies, Xu *et al.* concluded that *B. longum* was more effective than *Lactobacillus acidophilus* in NAFLD and that these beneficial effects were related to a modification of the gut microbiota [66]. Our own studies have shown that the modulation of the enteric microbiota can influence the fatty acid composition of host tissues, including the liver, adipose tissue, and brain [67,68]. Thus, we observed a significant elevation in $c9$, $t11$ conjugated linoleic acid (CLA) in the livers of mice and pigs, after feeding with linoleic acid in combination with the probiotic organism *Bifidobacterium breve* NCIMB 702258. CLA is a microbial metabolite that has been associated with the alleviation of NAFLD [69]. Indeed, it is noteworthy that the VSL#3 probiotic cocktail has been shown to produce CLA *in vitro*, and the $c9$, $t11$ CLA isomer, in particular [70] Prebiotic preparations have also been shown to ameliorate liver inflammation in obese mice through a glucagon-like peptide-2 (GLP-2)-dependent effect on the gut barrier [71] and hold promise for the management of NAFLD and related disorders [72].

Alcoholic liver disease: In an animal model of alcoholic liver disease, Forsyth *et al.* found that *Lactobacillus rhamnosus* GG (LGG) could reduce liver fat content, necro-inflammatory score and myleoperoxidase expression. Interestingly, these beneficial effects correlated with a reduction in, or normalization of, intestinal permeability [73]. Indeed, the concept that a significant component of the beneficial effect of probiotics in alcohol-related liver injury is exerted at the level of the intestinal barrier is supported by other studies [74], as well as by a considerable literature attesting to important effects of probiotics on gut tight junctional and transcellular permeability in diverse disease models. Indeed, Wang *et al.* have provided a molecular basis for the action of the probiotic *L. rhamnosus* GG, one of the most widely studied organisms in this context, by demonstrating that the restoration of barrier function, in alcohol-induced liver injury, was mediated through the recovery of hypoxia-inducible factor (HIF) and trefoil factor in gut epithelial cells [75].

Complications of liver disease: A variety of probiotic preparations have been studied in relation to the various complications of liver disease. While *Lactobacillus*-fermented milk supplements failed to reduce bacterial translocation in an experimental model of prehepatic portal hypertension [76], in contrast, in a model of acute liver injury, Adawi *et al.* found that rectal administration of a number of *Lactobacillus* species, with or without arginine supplementation, led to a reduction in bacterial translocation. In this same model, *Lactobacillus plantarum* alone abrogated the severity of the liver injury [77]. In an animal model of minimal HE, Jia and Zhang showed that a probiotic cocktail was as effective as lactulose in reducing HE and blood ammonia levels as well as decreasing endotoxaemia, liver injury and inflammation [78].

Review

Effects on acute liver injury and chronic liver disease: Probiotics have been shown to reduce the impact of a variety of forms of acute liver injury [79–83], as well as the severity of more chronic forms of liver disease, such as that related to total parenteral nutrition (TPN) [84].

Clinical

Given all that has been reviewed above, it should come as no surprise that here is an ever-increasing interest in the potential therapeutic value of probiotics in a variety of contexts in relation to the liver and its diseases [61,85,86]. These include: HE, translocation and infectious complications, NAFLD/NASH and the prophylaxis of infections after liver transplantation [86]. In attempting to assess the relevant literature, the reviewer is confronted, not only by the issues that bedevil the interpretation of animal studies (varying probiotic strains and preparations, different animal models and outcomes), but also by limitations in study size and design, as well as variations in probiotic dose, formulation, route and duration of therapy. There is also limited data on the microbiological impact of probiotics on the abnormal microbiota that has been described in cirrhosis and other liver diseases [87]. While probiotics are generally assumed to be safe, there have been reservations regarding their use in immunosuppressed individuals; whether these preparations offer a greater risk of initiating septic complications among individuals with liver disease, given the presence of both portal-systemic shunting and impaired immune function (be it related to underlying disease or iatrogenic), is unclear and safety assessments have been limited [86].

NAFLD/NASH: A Cochrane Collaboration Systematic Review published in 2007, while failing to identify any randomized clinical trials, concluded that preliminary data from two pilot non-randomised studies suggested that probiotics may be well tolerated, improve conventional liver function tests and decrease markers of lipid peroxidation in NAFLD/NASH [88]. Their review included one study employing *L. Bulgaricus*, which produced a reduction in transaminases [89], and another with *L. rhamnosus* GG, which resulted in lower levels of alanine transaminase [90].

Alcoholic liver disease: Kirpich *et al.* randomized 66 Russian males with alcoholic psychosis to 5 days of therapy with a combination of *Bifidobacterium bifidum* and *L. plantarum* or standard therapy. At baseline, alcoholics had fewer bifidobacteria, lactobacilli and enterococci; however, after 5 days of probiotic therapy, bifidobacteria, and lactobacilli increased and, when checked 7 days after the initiation of probiotic therapy, alanine transaminase levels had declined significantly in the probiotic but not in the control group [91].

Complications of liver disease: Despite the presence, in the literature, of several individual trials attesting to the benefits of various probiotic preparations in HE and minimal/subclinical HE [92–95], a recent Cochrane Systematic Review concluded that, while probiotics appeared to exert a significant effect on blood ammonia levels, problems with trial interpretation (related, in turn, to shortcomings in study design) did not allow them to conclude that probiotics were effective against clinically meaningful end points [96]. It should be noted, however, that a separate systematic review that focused exclusively on minimal HE concluded that prebiotics, probiotics, and synbiotics were effective for this indication, but that lactulose (itself a prebiotic) was superior to both probiotics and synbiotics [97]. Similar issues limit one's ability to draw conclusions and generate recommendations regarding the role of probiotics in preventing infectious complications of liver disease. While reported effects of probiotics on modulating the microbiota [86,98], reducing levels of endotoxemia [98], normalizing impaired neutrophil function [99] and reducing levels of pro-inflammatory cytokines [100] could well translate into clinical benefit, this has yet to be demonstrated. The efficacy of lactulose, a prebiotic, and antibiotics, such as neomycin, paromomycin, and rifaximin, in HE, has been well documented. A recent meta-analysis suggested that the efficacy of rifaximin was similar to that of lactulose and older antibiotic regimes but associated with less adverse events [101]. There have been limited studies of probiotics in other complications; the probiotic cocktail VSL#3 had no effect on portal pressure in either compensated or very early decompensated cirrhosis [102].

Effects on acute liver injury and chronic liver disease: Data on the impact of probiotics on the evolution or natural history of liver disease, *per se*, is very limited. Though uncontrolled studies suggest that probiotics may reduce SIBO in IFALD, there is, as yet, no evidence that their administration can prevent the development or progression of this form of liver disease [61]. Similarly, small studies in compensated cirrhosis [103] and PSC [104] showed no impact from the relatively short-term (3–6 months) administration of probiotic on common laboratory parameters. While probiotic supplementation has been shown to reduce a biomarker of risk for hepatocellular cancer [105], the actual clinical impact of this approach is unknown.

Conclusions and future studies

A central role for the microbiota in the precipitation of infectious and non-infectious complications of liver disease has been established and evidence for a more fundamental role in the aetiology of certain liver diseases, such as NAFLD and NASH, continues to accumulate. However, the description of the microbiota and its metabolic [106] and immunological [107] functions in various forms and stages of liver disease is still far from complete, but should be nigh given the widespread availability of high-throughput sequencing and metabolomic technologies. While high quality clinical evidence supports the use of antibiotic therapy, presumably as a modulator of the microbiota, in the management of HE, SBP, and other infectious complications, how these interventions impact on the microbiota and microbiota-host interactions has not been clearly defined. While probiotics hold promise in the management of liver disease and their potential is supported by a considerable volume of laboratory work [108], high-quality clinical evidence is scanty and its paucity, together with all of the quality control, strain selection and dose optimization issues that have been an unfortunate feature of this field, precludes, for the moment, recommendations on the use of probiotics, in general, and specific strains, in particular, in clinical practice. However, there is a sufficiently strong rationale for the use of strategies that involve the modulation of the microbiota in the management of liver diseases, such as NAFLD [109–111], and their complications to suggest that these approaches are deserving of further exploration.

Conflict of interest

The authors declared that they do not have anything to disclose regarding funding or conflict of interest with respect to this manuscript.

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Review