Intestinal virome and therapeutic potential of bacteriophages in liver disease

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Summary
Humans harbour a large quantity of microbes in the intestinal tract and have evolved symbiotic relationships with many of them. However, several specific bacterial pathobionts are associated with liver disease pathogenesis. Although bacteriophages (phages) and eukaryotic viruses (collectively known as “the virome”) outnumber bacteria and fungi in the intestine, little is known about the intestinal virome in patients with liver disease. As natural predators of bacteria, phages can precisely edit the bacterial microbiota. Hence, there is interest in using them to target bacterial pathobionts in several diseases, including those of the liver. Herein, we will summarise changes in the faecal virome associated with fatty liver diseases and cirrhosis, and describe the therapeutic potential of phages and potential challenges to their clinical application.

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Introduction
Work over the last few decades has increasingly shed light on the myriad of ways in which the different microbial communities that colonise our guts influence human health and disease.1 These microbial communities are composed of bacteria, fungi, viruses, and archaea that together encode >100-fold more genes than the human genome.2 The composition of the gut microbiome is significantly influenced, even from birth, by our surrounding environment and these encoded genes in turn have the potential to be of both benefit and harm.3 Most of our understanding of how the gut microbiota affects human disease has been focused on bacteria, but with new advances in metagenomic methods, viruses are beginning to receive more attention.

About 90% of the human intestinal virome is made up of bacteriophages (phages or prokaryotic viruses), while the other 10% are eukaryotic vi- ruses (Fig. 1).4 Eukaryotic DNA and RNA viruses include plant and mammalian viruses, some of which can use intestinal cells as their host. Some eukaryotic viruses can affect human health by causing disease, like the well-known enteric pathogens Norovirus, Rotavirus, and Enterovirus, while others are not pathogenic. Plant viruses are likely derived from the diet.5 The phageome consists of approximately 1015 bacteriophages and is largely composed of the order Caudovirales (family Siphoviridae, Myoviridae, and Podoviridae) and family Microviridae.6 In healthy individuals, the intestinal viral microbiome exhibits a high level of interpersonal heterogeneity with relative intrapersonal stability.7,8 However, changes in the virome community can be seen with changes in lifestyle such as diet and with different disease states.6,9 Deep sequencing of the intestinal viral microbiome in healthy individuals suggests that there is a small core group of phages shared among the majority of people, with a wider range of rarer phages that are unique to individuals.6,9 Understanding how the composition of the intestinal viral microbiome differs amongst individuals with different disease states will help us elucidate the mechanism by which the viral microbiome influences disease. Differences in the viral microbiome have already been implicated in the pathogenesis of obesity, type 2 diabetes, colon cancer, inflammatory bowel disease, and more.

Herein, we will review current literature focused on the human intestinal virome in liver disease. An estimated 1.5 billion people have chronic liver disease worldwide and an estimated 1.2 million patients with cirrhosis will die every year, making it one of the leading causes of death globally.10 Our existing strategies for reversing or preventing progression of liver disease are limited and often liver transplantation is the only therapy available to patients once they progress to end-stage liver disease. In recent years, we have improved our understanding of how the intestinal microbiome contributes to liver disease and with that, there is increased interest in targeting the intestinal microbiome to treat liver disease. Hence, we will also review the use of phage therapy in...
gastrointestinal and liver diseases and summarise the key bacteria that may serve as potential targets for phage therapy in the future.

**Intestinal virome in patients with liver disease**

Both eukaryotic viruses and bacteriophages have been implicated in liver disease pathogenesis. Eukaryotic viruses include the known pathogenic and hepatotropic viruses, hepatitis A (*Picornaervidae* family) and E (*Hepeviridae* family), which can be transmitted by the oral-faecal route and detected in the stool. Both HAV and HEV exist in two forms, a non-enveloped form comprised of a capsid surrounding the RNA genome and a quasi-enveloped form that is masked within a layer of the host cell membrane.\(^{12}\) The non-enveloped form, which is found in the stool and saliva of infected individuals, can survive harsh conditions such as transit through the gastrointestinal tract and cross the intestinal barrier into the blood via mechanisms that are not well understood. Recent work suggests that once in the blood, HAV harnesses endosomal gangliosides to infect hepatocytes and Kupffer cells,\(^{13}\) where it replicates and exits back into the bloodstream in its quasi-enveloped form, which camouflage its antigenic proteins from neutralising antibodies.\(^{14}\) Other known eukaryotic viruses that can be found in the intestinal virome and cause liver injury include Epstein-Barr virus (EBV), cytomegalovirus, and severe acute respiratory syndrome coronavirus 2.\(^{15–17}\)

Recent work by Jiang *et al.* investigating the intestinal virome in patients with alcoholic hepatitis and alcohol use disorder demonstrated that variations in intestinal viral taxa are associated with disease severity and mortality.\(^{18}\) Comparing the intestinal viromes of patients with alcoholic hepatitis, alcohol use disorder, and controls, faecal samples from patients with alcohol use disorder had significantly higher viral diversity and richness compared with controls, and this was generally correlated with lower bacterial diversity. In patients with alcoholic hepatitis, *Escherichia, Enterobacteria*, and *Enterococcus* phages were overrepresented compared to controls, while *Parabacteroides* phages were underrepresented. Further, an increased abundance of *Staphylococcus* phages and *Citrobacter* phages were associated with increased disease severity.

Aside from differences in phage composition, faecal samples from patients with alcoholic hepatitis also contained significantly more mammalian viruses, such as those from the *Parvoviridae* and *Herpesviridae* families, than controls. *Herpesviridae* was only present in faecal samples from patients with alcoholic hepatitis, with most of the assigned reads attributed to EBV.\(^{18}\) It is unclear why EBV is only detected in the guts of patients with alcoholic hepatitis, though a possible hypothesis is suppression of immunosurveillance in these patients. Alternatively, EBV reactivation might induce the development of hepatitis in alcoholic patients. Notably, a study of the intestinal virome in patients with non-alcoholic fatty liver disease (NAFLD) did not observe increased proportions of mammalian viruses compared to controls.\(^{19}\) Further studies are needed to confirm and characterise the intestinal mammalian virus population in patients with alcohol-associated liver disease as this may shed light on its pathogenesis.

Another difference noted between NAFLD and alcohol-associated liver disease is that patients with NAFLD and fibrosis had significantly lower intestinal viral diversity and proportionately fewer phages compared with controls.\(^{19}\) Incorporating faecal viral diversity in addition to clinical data into a model to non-invasively predict histologic fibrosis severity significantly improved the model’s diagnostic accuracy. Additionally, the abundance of several *Lactococcus* and *Lactobacillus* phages were inversely correlated with severity of liver fibrosis, whereas the abundance of *Lactobacillus* phages was positively correlated with the severity of liver fibrosis. Though the abundance of some phages was inversely correlated with their respective bacterial hosts, viral diversity did not correlate with bacterial diversity. It is difficult to draw conclusions regarding how liver disease affects the phage/bacteria relationship with data from a single timepoint.

One study evaluated phage/bacteria interactions across 2 timepoints in patients with compensated cirrhosis before and after 8 weeks of treatment with rifaximin.\(^{20}\) This study reported a significant reduction in the genus-level richness of the bacterial but not viral population after rifaximin use. Decreased complexity of bacterial-phage interactions was also seen after rifaximin, with complete collapse of bacterial-phage interactions seen in phages directed against pathobionts such as *Streptococcus, Pseudomonas*, and *Enterobacteriaceae* spp. These changes are most likely secondary to the direct impact of rifaximin on the bacterial population, and it is unclear how much cirrhosis contributed to these dynamics. Cross-sectional analysis revealed that phage-bacterial correlation network complexity was highest in controls, lowest in patients with cirrhosis taking only lactulose, and improved in patients taking both lactulose and rifaximin.\(^{20}\) A notable technical difference between these studies is that this study performed metagenomic sequencing of faecal DNA whereas the prior 2 studies used filtration techniques to isolate RNA- and DNA-containing viral particles from stool, followed by metagenomic sequencing.

Research on the intestinal virome is in its infancy and a causative link between changes in the

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**Key point**

Patients with liver disease exhibit differences in both the diversity and composition of their intestinal viromes compared with healthy controls, though the impact of these differences on the bacterial microbiome requires further study.
phageome and disease has not been established. It remains to be seen whether changes in the virome are disease drivers, or whether they are the result of disease. Future longitudinal studies are required to confirm virome changes in independent cohorts of patients, and to test the stability of the faecal virome and its correlation with liver disease severity over time. The analysis of the virome depends on metagenomic sequencing, methods for virome research have not been standardised, and only a small fraction of all sequences can be assigned to known viral taxa in public databanks. Improvements in bioinformatic analysis will lead to a better understanding of the dynamics of phage-bacteria interactions. This will allow us to answer the question of whether changes in phages drive bacterial dysbiosis or vice versa.

**Phage biology and their therapeutic potential**

**Bacteriophages - natural predators of bacteria**

Phages are viruses that infect bacteria and are considered to be the most numerous group of viruses on the planet, with an estimated $10^{31}$ total phage particles. Shortly after the discovery of phages by Frederick Twort in 1915, they were used to treat bacterial haemorrhagic dysentery. Phage therapy is the practice of using preparations of infectious phages to treat bacterial infections, which has the advantage over antibiotics of targeting specific bacterial species or strains while self-replicating and spreading to infect additional target bacterial cells. Phage therapy became very popular throughout the world to treat a wide range of diseases caused by both Gram-positive and Gram-negative pathogens, such as *Staphylococcus*, *Streptococcus*, *Vibrio*, *Klebsiella*, *Enterobacter*, *Shigella*, *Escherichia*, *Pseudomonas* and *Providencia* to name a few.

Commercial production of phage cocktails was initiated in France by what would later become L'Oreal, followed by the Eliava Institute of Bacteriophage, Microbiology and Virology (EIBMV) (Tbilisi, Georgia) and the Hirsfeld Institute of Immunology and Experimental Therapy (HIET) (Wroclaw, Poland). In the US, pharmaceutical giant Eli Lilly (Indianapolis, IN) produced 7 phage cocktails.

After the discovery and use of antibiotics, phage therapy fell out of favour in many Western countries, particularly the US. Much of the concern regarding the efficacy of phages as a therapeutic stemmed from reproducibility issues where the same successful cocktail of phages used on one patient did not work for all patients. This was presumably due to the narrow host range of the selected phages. Another problem was inflammatory responses to the phage cocktail. This was more likely due to contamination of the lysate by bacterial endo and exotoxins used to grow the phages in production rather than an immune response to the phages. Likewise, there was concern that the rapid lysis of cells by phage-encoded lytic enzymes can cause septic shock; however, this argument also applies to bactericidal antibiotics.

Phages come in all shapes and sizes, with genomes consisting of either double-stranded or single-stranded DNA or RNA. They were originally defined based on morphology and categorised into 21 morphotypes, before nucleic acid sequencing technologies started being used for taxonomic classification. Phages can be tailed, polyhedral, filamentous, pleomorphic, enveloped or not, but it is the double-stranded DNA tailed phages in the order *Caudovirales*, that are both the most common and the most commonly used for therapeutic

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**Fig. 1. Intestinal virome.** The intestine and liver are intimately connected and communicate via the portal vein and the common bile duct. The intestinal microbiota contains bacteria, fungi, archaea and viruses. Viruses in the intestinal virome are predominantly phages (also called phageome), but also contain some eukaryotic viruses. Lytic phages can lyse bacteria and contribute to changes in the bacterial microbiota. Graphic illustration was created in Biorender.com.
purposes. In addition to morphotype and nucleic acid type, there are 2 main lifestyle categories of phages, lytic (a.k.a., virulent, obligately lytic) and temperate. Lytic phages can replicate only via the lytic life cycle that ends with the destruction of the infected bacterial cell and release of progeny phages (Fig. 2A) while temperate phages can choose between the lytic and lysogenic life cycles (Fig. 2B). The latter includes the integration of phage DNA into the bacterial chromosome and its passive replication.26–30 Temperate phages show reduced lytic abilities, may incorporate and transfer (i.e., transduce) bacterial DNA, including drug resistance and pathogenicity genes, and can convert a bacterium into a “lysogen” (i.e., bacterial cell with a viral genome integrated into the bacterial chromosome) that becomes immune to superinfection by the same phage or related phages. Therefore, temperate phages have historically not been used as therapeutics.31 However, temperate phages can be modified to become obligately lytic32,33 and since temperate phages are commonly found in bacteria, their modification could expand the arsenal of therapeutic phages, at least for some pathogens with limited or no known lytic phages.34

Although the host range of a phage tends to be quite narrow (e.g., strain/serotype- or species-specific),35 there are lytic phages that can infect more distantly related bacteria.36 Host specificity is largely determined by receptor-binding proteins, a.k.a. anti-receptors.37–43 These phage-encoded proteins enable high affinity binding of phage virons to receptors located on the outer surface of bacterial cells, such as lipopolysaccharide, lipoteichoic acid, capsular polysaccharide, flagella and pili.44 Swapping domains or altering the sequence of specific regions of receptor-binding proteins results in altered host range specificity, enabling the phage to attach to different strains or different bacterial genera.45–48 Phages typically encode a single receptor-binding protein, but can encode more than one, resulting in polyvalency (i.e., the ability to bind to more than 1 receptor and potentially more than 1 host organism/strain).49

Resurgence of phage therapy
The widespread overprescribing of antibiotics by physicians coupled with the overuse of antibiotics in the livestock industry are key factors that are thought to have led to the global spread of antibiotic-resistant bacteria.49,50 This poses a serious public health problem since there are few, or in some cases, no drugs available to treat life-threatening bacterial infections. Antibiotics are not entirely safe either, as they can cause allergic reactions and severe side effects, including organ damage and a clearing of the normal commensal gut microbiota, leaving the gut vulnerable to secondary infections by opportunistic pathogens such as Clostridiodes difficile.51 Because phages are host specific rather than broad spectrum like many antibiotics, phage therapy has the potential to have fewer off-target effects on beneficial bacterial microbiome species. Phages are now being used to treat livestock infections,3–55 to prevent food spoilage,56–61 in human compassionate use cases, and in clinical trials.

Phage therapy in gastrointestinal diseases
Over the last 2 decades, several clinical trials have been performed with T4-like phages (Table 1). Oral administration of phage cocktails is considered safe in both healthy adults and children, with only occasional side effects independent of phage dosage.62–65 To determine the safety and efficacy of phage therapy for gastrointestinal infections, a T4-like coliphage cocktail was given orally for 4 days to children hospitalised with acute diarrhoea. Non-bacterial causes of diarrhoea were not ruled out. No adverse events were reported, suggesting the overall safety of the phage cocktail.64 However, substantial intestinal replication of phages was not observed, and phage treatment did not lead to an improvement in quantitative diarrhoea parameters, such as stool output and frequency, compared to placebo.66 This could be explained by the fact
that only half of patients actually harboured *Escherichia coli* (*E. coli*) strains susceptible to the administered phages, and *E. coli* only represented 5% of total faecal bacteria. Overall, this trial confirmed the safety of phage treatments in children with diarrhoea. Though the trial failed to show efficacy, this could potentially be explained by low *E. coli* abundance in the stool samples or symptoms caused by a non-bacterial infection (e.g. viral gastroenteritis).

One successful case was reported in 2016, in which a 68-year-old male patient was suffering from necrotising pancreatitis complicated by a pancreatic pseudocyst infected with multidrug-resistant *Acinetobacter baumannii*. Phages were applied by intracavitory and intravenous routes, and the patient completely recovered after 5 months.

Although this is only a case report, the obvious clinical improvements suggest that phage therapy might be useful for treating bacterial infections, and especially those caused by multidrug-resistant bacteria.

Phage therapy may also be a promising way to precisely edit the gut microbiota. Two randomised, placebo-controlled trials have been performed to determine the safety and efficacy of phages in adults suffering from mild to moderate gastrointestinal distress (e.g., gas, bloating, diarrhoea, constipation, etc) (NCT03269617; NCT04511221). Over the 28-day study, oral administration of the coli-phage cocktail was shown to be safe and well tolerated. Patients experienced a similar reduction in gastrointestinal symptom severity during both the treatment and placebo periods, suggesting that the phage therapy was ineffective, but there was also no evidence that patients’ initial symptoms were secondary to overgrowth of the bacteria targeted by the administered phage. Future studies can evaluate the efficacy of phage therapy by documenting the interactions of phages in these cocktails with the specifically targeted bacterial strains obtained from treated patients.

In addition to these clinical trials, there are also some encouraging preclinical data (Table 1). Adherent-invasive *E. coli* (AIEC) have been shown to be involved in the pathogenesis of inflammatory bowel diseases. Administration of a phage cocktail against these *E. coli* strains reduced intestinal AIEC colonisation in transgenic mice expressing the human AIEC receptor. Furthermore, wild-type mice colonised with AIEC were protected from dextran sodium sulphate-induced colitis upon phage treatment, with less *E. coli* in faeces, as well as in ileal and colonic sections. To evaluate the ability of the phage cocktail to target AIEC strains in patients, ileal biopsies from patients with Crohn’s disease were spiked with an AIEC strain. Active phage replication was detected 5 hours and 24 hours after phage administration, confirming the killing potential of phages in such an environment. A phase I/IIa placebo-controlled clinical trial was therefore initiated, to assess the safety and efficacy of the phage cocktails in patients with inactive Crohn’s disease (NCT03808103).

### Potential for phage therapy in liver disease

**Known bacterial pathobionts driving liver disease as potential targets for phage therapy**

Our existing knowledge of the taxonomic differences in the bacterial microbiota of patients with liver disease can help guide further investigation into potential targets for phage therapy. In the following subsections, we summarise faecal bacterial changes in selected human liver diseases (Table 2).

#### Non-alcoholic fatty liver disease

NAFLD encompasses a spectrum of diseases ranging from excessive fat deposition in the liver in the absence of significant alcohol use (simple steatosis or NAFL) that can progress to liver inflammation (non-alcoholic steatohepatitis [NASH]), and eventually fibrosis. Several studies have found a decreased faecal abundance of *Faecalibacterium* and specifically *Faecalibacterium prausnitzii* in both obese and non-obese patients with NASH. *Ruminococcus* was enriched in obese patients with NASH in 1 study, but reduced in other studies of obese and non-obese patients. *Ruminococcus obeum* was specifically found to be reduced in patients with NAFLD in 1 study. Advanced fibrosis secondary to NASH is associated with an overall decrease in intestinal bacterial diversity and an increase in the relative abundance of Gram-negative bacteria such as *Bacteroides* and *Escherichia*. Although no causative role of these bacterial strains for steatohepatitis has been demonstrated in preclinical models, Yuan *et al.* demonstrated that an ethanol-producing *Klebsiella pneumoniae* (*K. pneumoniae*) strain was present in 60% of a Chinese cohort of patients with NAFLD and that introducing this strain into mice induced steatohepatitis.

#### Alcohol-associated liver disease

Heavy alcohol use leads to a spectrum of liver diseases beginning with steatosis, which can be reversible or can progress to steatohepatitis and fibrosis in susceptible patients. Studies of the intestinal bacterial microbiome in patients with alcohol-associated liver disease have revealed enrichment of *Enterobacteriaceae* and a reduction of *Lactobacillus*, *Bacteroides*, *Bacteroides*, *Akkermansia*, and *Enterococcus faecalis* (*E. faecalis*). A recent study by Duan *et al.* demonstrated that patients with alcoholic hepatitis have an increased relative abundance of *Enterococcus faecalis* (*E. faecalis*) and specifically a strain that secretes the exotoxin cytolsin. The presence of cytolsin-secreting *E. faecalis*
Table 1. Overview of recent studies of phage-related treatment in gastrointestinal diseases. (English literature only).

<table>
<thead>
<tr>
<th>Type of study</th>
<th>Phage (target)</th>
<th>Dose and method</th>
<th>Patients</th>
<th>Result and conclusion</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical trial</td>
<td>Phage T4 (E. coli)</td>
<td>10^7 PFU/ml, dose A, 10^5 PFU/ml, dose B, Oral administration</td>
<td>15 healthy individuals</td>
<td>Safe, gut microbiota profile not affected</td>
<td>62</td>
</tr>
<tr>
<td>Clinical trial</td>
<td>T4-like Phages (E. coli)</td>
<td>3x10^6 PFU/ml, dose A, 3x10^7 PFU/ml, dose B, Oral administration</td>
<td>15 healthy individuals</td>
<td>Safe, but E. coli abundance not changed</td>
<td>63</td>
</tr>
<tr>
<td>Clinical trial</td>
<td>Commercial phage cocktail ColiProteus (E. coli)</td>
<td>Oral administration</td>
<td>5 healthy adults, 10 healthy children</td>
<td>Overall safe, with occasional reported side effects independent of dosage</td>
<td>64</td>
</tr>
<tr>
<td>Clinical trial</td>
<td>T4-like Phages or ColiProteus (E. coli)</td>
<td>10^7 or 10^5 PFU for older children (T4-like phages), 10^7 or 10^5 PFU for younger children (T4-like phages), 5x10^8 or 10^9 PFU for all (ColiProteus), Oral administration</td>
<td>20 older children, 20 younger children</td>
<td>Both cocktails are safe</td>
<td>65</td>
</tr>
<tr>
<td>Clinical trial</td>
<td>T4-like Phages or ColiProteus (E. coli)</td>
<td>3.6x10^8 PFU (T4-like phages), 1.4x10^9 PFU (ColiProteus), Oral administration</td>
<td>120 children with diarrhoea</td>
<td>Safe, but lack of efficacy</td>
<td>66</td>
</tr>
<tr>
<td>Clinical case report</td>
<td>Phage cocktail (A. baumannii)</td>
<td>5x10^7 PFU Oral administration</td>
<td>68-year-old male with necrotising pancreatitis complicated by pancreatic pseudocyst</td>
<td>Patient completely recovered</td>
<td>67</td>
</tr>
<tr>
<td>Clinical trial</td>
<td>Phage cocktail PreforPro (E. coli)</td>
<td>One 15 mg capsule, Oral administration</td>
<td>32 healthy individuals with mild to moderate gastrointestinal distress</td>
<td>Safe and tolerable, but no difference from placebo</td>
<td>68</td>
</tr>
<tr>
<td>Clinical trial</td>
<td>Phage cocktail PreforPro (E. coli), together with probiotics Bifidobacterium animalis subspecies lactis strain BLO4</td>
<td>Oral administration</td>
<td>68 healthy individuals with mild to moderate gastrointestinal distress</td>
<td>Safe and tolerable, but no compelling evidence of efficacy</td>
<td>69</td>
</tr>
<tr>
<td>Preclinical study</td>
<td>Phage cocktail (adherent-invasive E. coli)</td>
<td>3x10^7 PFU Oral administration</td>
<td>Wild-type mice colonised with 10^8 CFU of adherent-invasive E. coli, Dextran sodium sulphate-induced colitis</td>
<td>Faecal E. coli level decreased; dextran sodium sulphate-induced colitis ameliorated</td>
<td>70</td>
</tr>
<tr>
<td>Preclinical study</td>
<td>Phage cocktail (E. faecalis)</td>
<td>10^10 PFU Oral administration</td>
<td>Gnotobiotic mice colonised with stool samples from cytolysin-positive patients with alcoholic hepatitis, Ethanol-induced liver disease</td>
<td>Faecal E. faecalis level decreased, ethanol-induced liver disease ameliorated</td>
<td>87</td>
</tr>
</tbody>
</table>

A. baumannii, Acinetobacter baumannii; CFU, colony-forming unit; E. coli, Escherichia coli; E. faecalis, Enterococcus faecalis; PFU, plaque-forming unit.

correlated with the severity of liver disease and with mortality in patients with alcoholic hepatitis, and oral administration of cytolysin-positive E. faecalis promotes ethanol-induced liver injury in mice.87

Autoimmune hepatitis
Autoimmune hepatitis is a chronic inflammatory liver disease whose pathogenesis is poorly understood, though genetic susceptibility and loss of tolerance against liver antigens are proposed mechanisms.88 Patients with autoimmune hepatitis have an overrepresentation of potential pathogens, including Veillonella species such as Veillonella disper, in their faecal microbiomes.89 Translocation of Enterococcus gallinarum (E. gallinarum) to the liver triggered an autoimmune response in mice genetically predisposed to autoimmunity. Subsequent antibiotic treatment prevented the formation of pathogenic autoantibodies and T cells, thus improving mortality.90 E. gallinarum DNA was detected in the livers of most patients with autoimmune hepatitis but in none of the healthy control livers.90

Primary sclerosing cholangitis
Primary sclerosing cholangitis (PSC) is a cholestatic liver disease characterised by inflammation of the bile ducts leading to stricturing and sclerosis and eventually progressive biliary fibrosis and cirrhosis.
Table 2. Bacterial genera and species with known correlations to different aetiologies of liver disease.

<table>
<thead>
<tr>
<th>Aetiology of Liver Disease</th>
<th>Increased</th>
<th>Decreased</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-alcoholic fatty liver disease</td>
<td>• Bacteroides 78,79,80,107,109</td>
<td>• Bacteroides 78,80</td>
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<tr>
<td></td>
<td>▫ Bacteroides vulgatus 76</td>
<td>▫ Bacteroides caccae 78</td>
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<td></td>
<td>▫ Biautia 78,109</td>
<td>▫ Bifidobacterium 73,74,111</td>
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<td></td>
<td>▫ Dorea 73,110</td>
<td>▫ Coprococcus 73,74,112</td>
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<td></td>
<td>▫ Escherichia 73,74,80,111</td>
<td>▫ Faecalibacterium 73,74,80,111</td>
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<td>▫ Lactobacillus 73,74,109</td>
<td>▫ Faecalibacterium prausnitzii 73,74,110</td>
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<td></td>
<td>▫ Klebsiella 73,110</td>
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<td></td>
<td>▫ Klebsiella pneumoniae 81</td>
<td>▫ Oscillatoria 73,112</td>
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<td></td>
<td>▫ Roseburia 119</td>
<td>▫ Roseburia 119</td>
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<tr>
<td></td>
<td>▫ Ruminococcus 73,79</td>
<td>▫ Ruminococcus 73,76,112</td>
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<tr>
<td>Alcohol-associated liver disease</td>
<td>• Biautia 80</td>
<td>• Akkermansia 85,114</td>
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<td></td>
<td>▫ Dorea 113</td>
<td>▫ Faecalibacterium 113</td>
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<td></td>
<td>▫ Enterococcus 87,114</td>
<td>▫ Faecalibacterium prausnitzii 113</td>
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<td></td>
<td>▫ Enterococcus faecalis 87</td>
<td>▫ Ruminococcus 113</td>
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<tr>
<td></td>
<td>▫ Prevotella 113</td>
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<td></td>
<td>▫ Veillonella 114</td>
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<td>Autoimmune hepatitis</td>
<td>• Enterococcus 80</td>
<td>• Prevotella 115</td>
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<td></td>
<td>▫ Enterococcus gallinarum 80</td>
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<tr>
<td></td>
<td>▫ Veillonella dispar 80</td>
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<tr>
<td>Primary sclerosing cholangitis</td>
<td>• Enterococcus 80</td>
<td>• Clostridium 93,95</td>
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<td>▫ Lactobacillus 80,95,96</td>
<td>▫ Coprococcus 92,94,95</td>
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<td>▫ Streptococcus 92,95,96</td>
<td>▫ Faecalibacterium 92,95,97</td>
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<td>▫ Veillonella 92,97</td>
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<td>Cirrhosis</td>
<td>• Enterococcus 85</td>
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<td>▫ Enterococcus faecalis 85</td>
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<td>▫ Streptococcus 2,79,85,117–119</td>
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Several recent studies compared the faecal bacterial microbiota of patients with PSC and healthy controls. Patients with PSC are consistently shown to have lower bacterial microbiome diversity than healthy controls. Additionally, Veillonella has been shown to be enriched in the stool of patients with PSC compared to healthy controls in multiple studies. Enterococcus, Streptococcus and Lactobacillus are also frequently enriched in patients with PSC, whereas there is a relative depletion of short-chain fatty-acid-producing Firmicutes, such as Faecalibacterium and Coprococcus. Germ-free mice inoculated with faecal matter from patients with PSC were more susceptible to hepato-biliary injury by diethyldithiocarbamate and harboured K. pneumoniae, Proteus mirabilis, and E. gallinarum in their mesenteric lymph nodes. Further, specific K. pneumoniae strains could induce pore formation on human intestinal epithelial organoids, suggesting that increased bacterial translocation could be a potential mechanism of increased susceptibility to hepato-biliary injury.

Cirrhosis
Patients with cirrhosis have decreased proportions of beneficial, autochthonous taxa, such as Lachnospiraceae and Ruminococcaceae, and overrepresentation of potentially pathogenic bacteria such as Enterobacteriaceae, Staphylococcaceae, and Enterococcaceae, whose abundance correlates with disease progression and endotoxemia 75,79,103. Another study observed a higher relative abundance of bacteria normally associated with oral flora in the intestinal microbial community of patients with cirrhosis, as well as increased Veillonella and Streptococcus species compared to controls. Changes in the composition of the intestinal bacterial microbiome have also been correlated with the severity of liver disease. The ratio of autochthonous taxa, such as Ruminococcaceae, Lachnospiraceae, and Clostridiales, to non-autochthonous taxa, such as Enterobacteriaceae and Bacteroidaceae, was much higher in healthy individuals than patients with cirrhosis and inversely correlated with model for end-stage liver disease score and degree of hepatic decompensation. Moreover, an increased relative abundance of pathogenic bacteria was associated with the development of complications such as hepatic encephalopathy.

Patients with cirrhosis not only exhibit an increased relative abundance of pathogenic bacteria in their intestinal bacterial microbiomes, they are also at increased risk of bacterial translocation, a process whereby bacteria migrate from the intestinal lumen to extraintestinal sites. Aerobic Gram-negative bacteria, such as E. coli, aerobic Gram-positive bacteria, such as Enterococcus faecalis, and anaerobic bacteria, such as Bacteroides vulgatus, are the most frequently isolated bacteria from the portal venous system in patients with liver cirrhosis. These bacteria are known to cause sepsis, hepatic encephalopathy, and other complications in patients with liver cirrhosis.

Key point
Our knowledge of taxonomic differences in the bacterial microbiomes of patients with different aetiologies of liver disease could help identify potential targets for phage therapy.
Enterococcus faecalis, Pseudomonas aeruginosa, and other Enterobacteriaceae, translocate much more readily than anaerobic bacteria.\(^7\) Notably, these species have also been implicated in decompensation of cirrhosis.\(^8\) Increasingly, studies are comparing patients with cirrhosis based on their disease aetiology. While many intestinal bacterial communities are shared across the spectrum of liver disease aetiologies, differences in the patterns of dysbiosis across the different aetiologies of cirrhosis may provide a better picture of the mechanisms underlying these associations.

### Preclinical phage utilisation in liver disease

Although no clinical trial using phage therapy for patients with liver disease has been published, 2 preclinical studies used phage therapy to treat liver disease. Duan et al. demonstrated that intestinal levels of *E. faecalis* are significantly increased in patients with alcoholic hepatitis.\(^9\) Furthermore, the presence of a specific strain of *E. faecalis* that produces the bacterial exotoxin cytolysin correlates with increased severity of disease and mortality in patients with alcoholic hepatitis. Transplantation of faeces from cytolysin-positive patients with alcoholic hepatitis worsened ethanol-induced liver disease in gnotobiotic mice, whereas treatment of these mice with specific phages targeting cytolytic *E. faecalis* by oral gavage, reversed the exacerbation of liver disease. No improvement in liver disease was seen in the gnotobiotic mice treated with phages targeting non-cytolytic *E. faecalis*. This preclinical study demonstrates the utility of targeting specific species of the intestinal bacterial microbiome to modify disease progression.

Another study showed that selective elimination of the ethanol-producing *K. pneumoniae* strain using phages prior to faecal transplantation into mice prevented development of diet-induced steatohepatitis.\(^10\) These studies are good examples of how elimination of pathobionts by phages can improve liver disease in mouse models.

### Conclusion and future directions

Recent advances in the field of microbiota research have identified a few bacterial strains that correlate with liver disease in patients, are causatively linked to disease pathogenesis, and could thus act as therapeutic targets. Despite the renewed interest in phage therapy, there are many roadblocks preventing it from becoming standard of care. One major roadblock is the narrow host range of a single virion.\(^11\) By targeting more than 1 receptor, we avoid many of the obstacles that bacteria have evolved to prevent phage adsorption (e.g., mutations to or physically blocking receptors with extracellular polysaccharides). Blocking receptors using biofilms can be avoided by expressing an extracellular polysaccharide-degrading enzyme in the phage.\(^12\)

Other obstacles to the widespread use of phage therapeutics relate to their pharmacokinetics and pharmacodynamics within the human body. For example, some phages administered systemically can be cleared rapidly from the circulation.\(^13\) It is therefore critical to determine the dose of phages being administered and their clearance from the site where they are applied, to ensure that phages are present at the specific site long enough to lyse bacteria.

Screening patients for the presence and sufficient abundance of target bacteria in the intestine, as well testing the susceptibility of target bacteria to phages, will be crucial for the successful clinical application of phage therapy. Combining a personalised treatment approach with phages’ ability to precisely edit the microbiota could make phage-based therapeutics powerful new tools for the treatment of many diseases, including those of the liver.

### Abbreviations

AIEC, adherent-invasive *E. coli*; *A. baumannii*, *Acinetobacter baumannii*; EBV, Epstein-Barr virus; *E. coli*, *Escherichia coli*; *E. faecalis*, *Enterococcus faecalis*; *E. gallinarum*, *Enterococcus gallinarum*; *K. pneumoniae*, *Klebsiella pneumoniae*; NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis; PSC, primary sclerosing cholangitis.

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### Conflicts of interest

B.S. has been consulting for Ferring Research Institute, HOST Therabiotics, Intercept Pharmaceuticals, Mabwell Therapeutics, Pataria Pharmaceuticals and Takeda. B.S.’s institution UC San Diego has received research support from Axial...
Biotherapeutics, BiomX, CymaBay Therapeutics, NGM Biopharmaceuticals, Prodigy Biotech and Synlogic Operating Company. B.S. is founder of Nterica Bio. UC San Diego has filed several patents with B.S. as inventor related to this work.

Please refer to the accompanying ICMJE disclosure forms for further details.

Authors’ contributions

C.H. was responsible for drafting the chapters “Introduction”, “Intestinal virome in patients with liver disease”, “Known bacteria driving liver disease as potential targets for phage therapy”, “Preclinical phage utilisation in liver disease”, coordinating the writing and compiling the final version. Y.D. was responsible for drafting the chapter “Phage therapy in gastrointestinal diseases”. D.E.F. was responsible for drafting the chapters “Bacteriophages - natural predators of bacteria”, “Resurgence of phage therapy and parts of “Conclusion and future directions”. B.S. was responsible for drafting the chapters “Brief Summary” and parts of “Conclusion and future directions”. All authors revised and approved the manuscript.

Supplementary data

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Author names in bold designate shared co-first authorship

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Review


