Review

Biliary Diseases from the Microbiome Perspective: How Microorganisms Could Change the Approach to Benign and Malignant Diseases

Cecilia Binda 1, Giulia Gibiino 1, Chiara Coluccio 1,*, Monica Sbrancia 1, Elton Dajti 2, Emanuele Sinagra 3,4, Gabriele Capurso 5, Vittorio Sambri 6,7, Alessandro Cucchetti 2,8, Giorgio Ercolani 2,8 and Carlo Fabbri 1

Abstract: Recent evidence regarding microbiota is modifying the cornerstones on pathogenesis and the approaches to several gastrointestinal diseases, including biliary diseases. The burden of biliary diseases, indeed, is progressively increasing, considering that gallstone disease affects up to 20% of the European population. At the same time, neoplasms of the biliary system have an increasing incidence and poor prognosis. Framing the specific state of biliary eubiosis or dysbiosis is made difficult by the use of heterogeneous techniques and the sometimes unwarranted invasive sampling in healthy subjects. The influence of the microbial balance on the health status of the biliary tract could also account for some of the complications surrounding the post-liver-transplant phase. The aim of this extensive narrative review is to summarize the current evidence on this topic, to highlight gaps in the available evidence in order to guide further clinical research in these settings, and, eventually, to provide new tools to treat biliary lithiasis, biliopancreatic cancers, and even cholestatic disease.

Keywords: biliary tract microbiome; cholangiocarcinoma; biliary cancer; cholangitis; oncobiome

1. Introduction: The Microbiome in Healthy Patients

The human microbiota is one of the densest, and quickly developing ecosystems [1]. The term “microbiota” usually defines the assemblage of living microorganisms present in certain environment [2]. However, as phages, viruses, plasmids, prions, viroids, and free DNA are usually not considered as living microorganisms [3], they do not belong to the microbiota. The term microbiome, as it was originally postulated by Whipps and colleagues [4], includes not only the community of the microorganisms but also their “theatre of activity” [5].

In healthy conditions, the microbiome bacteria interact with the epithelial barrier and with the immune system influencing their feedback, but they are also able to produce
substances that influence the local metabolism, thus maintaining homeostasis [6,7]. For example, the normal gut microbiome has a specific function in host nutrient metabolism, xenobiotic and drug metabolism, maintenance of the structural integrity of the bowel mucosal barrier, immunomodulation, and defense from pathogens [8]. Therefore, an imbalance of the gut microbiome, due to antibiotics or to bacterial translocation, can promote the development of diseases [7].

The gastrointestinal system is one of the largest storing places of microorganisms in the human body and it holds both commensal and pathogenic microbial species [5,9]. To date, little is known about the composition of the biliary tract microbiota and its influence on the development of biliary diseases. In fact, bile represents a biological fluid produced in the liver, stored in the gallbladder (interdigestive), and successively released into the duodenum after food ingestion [10]. While the microbial species of different parts of gastrointestinal system have been extensively investigated in health and disease, the identification of bile microbiota has not been addressed [10]. Furthermore, the few available data on the biliary microbiota are limited to experimental animal and human pathological models [11].

In their experimental study, Jiménez and co-workers analyzed the bile, gallbladder mucus, and mucosal microbiome of healthy pigs using both culture-based and metagenomics techniques [10]. All the cultured samples harvested bacterial species and the number of identified species ranged from 3 to 20 per sample. All the bacteria isolated from cultures were broadly balanced among the Firmicutes (34%), Actinobacteria (32%) and Proteobacteria (32%) phyla. Bacteroidetes accounted for only a smaller part (2% of the isolates), thus highlighting an inadequate adaptation to the biliary environment. On the other hand, at the genus level, Staphylococcus, Streptococcus, Kocuria, Rothia, Acinetobacter, and Psychrobacter were isolated from different samples, suggesting their possible role as members of the core biliary microbiota of pigs [10]. Interestingly, the microbiological analysis of gallbladder mucus and mucosa broadened the spectrum of bacteria present in the bile that could possibly colonize these niches [10].

As aforementioned, research on the composition of the “healthy” human biliary microbiota is challenging. Indeed, bile sampling techniques, such as endoscopic retrograde cholangiopancreatography (ERCP), percutaneous biliary drainage, and surgical sampling, are invasive procedures that are typically performed when a biliary tract disease is diagnosed or suspected.

In the study performed by Molinero et al., the biliary microbiota of 27 liver donors (13 without and 14 with cholelithiasis) were analyzed [12]. The 16S ribosomal RNA sequencing showed a prevalence of Actinobacteria, Firmicutes and Bacteroidetes in both the bile samples and gallbladder tissues of subjects without gallstones [11,12]. Furthermore, a substantial increase in the presence of the Propionibacteriaceae family and Sphingomonas genus was also reported, when compared with individuals with gallstones [12]. This study provided evidence regarding the human biliary microbiota in healthy subjects. However, the study had limitations [2]. In fact, since liver donors undergo specific treatments and procedures, such as antibiotics, in the hospital’s intensive care units, their bile samples can hardly be considered “normal biliary microbiome”. In any case, these results give access to new perspectives in the identification of bacterial functions in a microbial ecosystem that was previously unexplored. Despite these interesting results, studies with a larger sample size are needed to confirm the findings. Indeed, the possible identification of a stable “biliary tract resident microbial community” may challenge the traditional knowledge on the development of biliary infectious diseases [11]. It may be tempting to hypothesize that a local dysbiotic process, rather than an ascending infection from the duodenum, better explains the occurrence of several biliary diseases, in a “microbiota-centric” view [11].

Recent insights into the biliary microbiota have improved the understanding of the pathogenesis of biliary diseases, such as gallstones, biliopancreatic cancer, and autoimmune cholangiopathies. The aim of this narrative review is to summarize the
available evidence about the role of the biliary microbiome in the onset of gallstone disease, biliary cancer, autoimmune cholestatic diseases and in some settings, such as in liver transplantation (LT).

2. The Microbiome and Gallstone Disease

Biliary stones are a leading cause of hospitalization in gastroenterological departments worldwide; in Europe the prevalence of gallstone disease is increasing more and more, ranging between 5.9% and 21.9% in large surveys [13]. The European Association for the Study of the Liver (EASL) reports that in Europe about 20% of inhabitants suffers of lithiasis [14].

The possible role of bacterial microorganisms in gallstone formation has gained growing interest over the last decade. Its pathogenesis, with variations between countries and regions, implies the synergic effect of genetic and environmental factors, such as ethnicity, gender, age, lithogenic genes, hypersecretion of cholesterol or bilirubin by the gallbladder or liver, bile stasis, diet, metabolism, intestinal factors, the use of drugs, lifestyle, and comorbidities [15–17]. Gutiérrez-Díaz and coworkers [18] confirmed the association between diet, biliary microbiota and gallstone disease, demonstrating that in patients with lithiasis, a modification in the abundance of bile microorganisms occurs. Bacteroidaceae and Bacteroides appear to be negatively correlated with dairy product intake, and Bacteroidaceae, Chitinophagaceae, Propionibacteraceae, Bacteroides, and Escherichia-Shigella are positively correlated with several kinds of fiber, phenolics, and fatty acids. Changes of the dynamic equilibrium among all these factors may influence the risk of lithiasis occurrence.

Several studies have suggested that a resident biliary tract microbiome exists with a high level of similarity with the duodenal one. The intestinal bacteria (Clostridium, Bifidobacterium, Peptostreptococcus, Bacteroides, Eubacterium, and Escherichia coli) involved in bile acid metabolism can interfere with enterohepatic circulation, leading to lithogenesis [19,20], in particular Firmicutes, Proteobacteria, and Bacterioidetes [21]. Moreover, pathogenic bacteria of the oral cavity can play a promoter role for gallstone onset, influencing the motility of the gallbladder and the expression of mucin genes (MUC1, MUC3, and MUC4) [22,23].

In 1966, Maki et al. highlighted the connection between bacterial infection and the formation of pigmented gallstones for the first time [24], demonstrating that the inoculation of bacterial β-glucuronidase in the bile could hydrolyze the bilirubin glucuronide into bilirubin and glucuronic acid, which, precipitating with calcium, forms calcium bilirubinate. Indeed, β-glucuronidase-expressing bacteria have been frequently identified in the samples of patients with pigmented gallstones [25,26]. Other bacterial enzymes, such as phospholipases and bile acid hydrolases have later been shown to be implicated with similar mechanisms in the formation of pigmented gallstones [27,28], reported as containing bacterial sequences of E. coli and Pseudomonas sp. [29]. The following studies have confirmed Maki’s hypothesis, making this theory widely accepted. Even if the formation of cholesterol gallstones has traditionally been considered to be affected by metabolic imbalances and genetic variances rather than a bacterial damaging effect [15], over the years a growing literature emerged, demonstrating that alterations of the gut microbiota also contribute to the formation of cholesterol gallstones [22,30,31], especially after the advent of innovative genomic techniques. Pseudomonas aeruginosa and Enterococcus faecalis have been reported to shorten the cholesterol crystallization time in the bile, suggesting that these species may be crucial in the formation of cholesterol gallstones [32].

The gut microbiota plays an active role in bile acid metabolism, regulating the size and composition of bile acids [12,33]. Changes in the bile acid pool represent a leading etiopathogenetic factor, as bile is composed mainly of bile acids (nearly 50%); cholesterol and fatty acids account for nearly 20%, while phospholipids and bilirubin account for a minority of bile [34,35].
The biliary and gut microbiota are involved in almost all passages of bile formation, such as the metabolism of lipid and cholesterol, biotransformation, and enterohepatic circulation. Indeed, a perturbation of the gut microbiota may influence bile acid homeostasis in any step of the host metabolic pathways, in particular regarding glucose and cholesterol metabolism, which is crucial for gallstone genesis [36]. We report the main microorganisms involved in this setting in the gut and biliary microbiota in Table 1.

While the biliary system in healthy patients was once considered to be sterile [37], it has been demonstrated that the gallbladder physiologically has a composite microbiota, with different possible routes leading to bacterial colonization of the biliary system, including duodenal translocation through the papilla or migration through blood vessels [38,39].

In 1995, Swidsinski et al. [40] analyzed the cholesterol gallstones from patients with negative bile culture using polymerase chain reaction (PCR)-based amplification and 16S ribosomal RNA sequencing, showing that bacterial DNA was present in 94% of mixed gallstones (containing cholesterol for 70–90%). Pure cholesterol gallstones (>90% of content) showed, instead, no bacterial DNA. Three groups of bacteria were identified: Propionibacteria-related, Clostridia-related, and Enterobacteria-related accounting for 45%, 35%, and 25% of the total, respectively.

While the high concentration of a single bacterial family is compatible with an infection, the concurrent presence of multiple bacterial species suggests a colonization. Several studies compared the biliary microbiota of patients with gallstones to those of other sites of the gastrointestinal tract with findings that all bacterial genera found in the biliary system were also retrieved in at least one other analyzed tract, thus supporting the case for bile colonization, which may be pivotal in lithiasis pathogenesis [41,42].

With the support of PCR, the presence of bacteria in the bile samples of patients affected by biliary tract disorders was discovered, with variable composition of Capnocytophaga spp., Lactococcus spp., Bacillus spp., Staphylococcus haemolyticus, Enterobacter or Citrobacter spp., Morganella spp., Salmonella spp., and Helicobacter pylori (HP) [42,43].

HP-produced urease represents a crucial link for gallstones through calcium precipitation [44]. One mechanism of action for HP is the release of proinflammatory and vasoactive substances, such as interleukins IL-1 and IL-6 and tumor necrosis factor (TNF)-alpha, which may cause oxidative stress and free radical release, that are related to gallbladder inflammatory disorders and to the cholelithogenesis [45,46].

The microbiota might also contribute indirectly to gallstone pathogenesis, affecting energy intake, bowel permeability, and supporting a chronic pro-inflammatory condition [47]. Several in depth studies investigated the metagenomic profiles of the different biliary bacterial communities. In patients with pigmented gallstones, genes extracted from Klebsiella and Enterococcus were found, which are thought to be involved in biofilm arrangement. Regarding cholesterol stones, bile resistance genes were selected from Escherichia, Shigella, Serratia, Bacillus, and Klebsiella [48].

Thanks to cultivation or PCR, several bacteria, such as Escherichia coli, Klebsiella pneumoniae, Enterococcus faecium, Enterobacter cloacae, and Pseudomonas aeruginosa, have been identified in bile or gallstone samples [49]. New methods, such as next-generation sequencing (NGS), helped us to significantly increase our knowledge regarding the microbial flora [50].

Wu and coworkers analyzed, in patients with cholesterol gallstone, gallbladder bile, gallstones, and feces by performing bacterial 16S rRNA amplicon sequencing [21]; they detected several gut bacterial operational taxonomic units (OTUs) in the biliary system and reported dysbiosis in the fecal samples from the patients with gallstones.

In a Chinese study, whole-metagenome shotgun (WMS) and 16S sequencing were used to investigate the bile samples of 15 patients with choledocholithiasis. Bile was collected from the common bile duct, and communities in the bile were compared to matched gut microbiota. Thirteen novel biliary bacterial species were identified in the human biliary tract that were never documented previously (such as P. piscicola and
Cellulosimicrobium cellulans), revealing heterogeneity among individuals and a prevalence of microorganisms from the oral cavity/respiratory system instead of the bowel. To note, in this study there was no correlation between previous endoscopic sphincterotomy and the biliary microbial composition, in contrast with the hypothesis that sphincterotomy increases the risk of biliary tract infection [51].

In the previously cited study of Peng et al., they used culture-dependent and culture-independent methods to study, in 22 patients affected by cholesterol gallstone, the composition and function of bacterial clusters in cholesterol gallstones and bile [42]. It was reported that Pseudomonas spp. were the dominant inhabitants in both groups. Moreover, its major role in the formation of cholesterol stones is supported by showing that 30% of the culturable strains were able to secrete β-glucuronidase and phospholipase A2, and that the Pseudomonas aeruginosa strains had the highest enzyme activity.

Others have reported that the prevalence of Pseudomonas, Bacillus, Klebsiella, Clostridium, Staphylococcus, and Enterobacter is more relevant in cholesterol gallstones than in the bile, supporting the hypothesis that a dysfunction of the bowel barrier with subsequent bacterial translocation into the biliary tract may act as a critical point in lithogenesis [52]. Of the previously reported genera, Escherichia, Brucella, Citrobacter, Shinella, Aurantimonas, Lachnospiraceae, and Lactobacillus were found in gallstones. Specifically, Citrobacter, Lactobacillus, and Aurantimonas are, indeed, common inhabitants of the gut. The presence of intestinal bacteria in cholesterol gallstones suggests that, after migration towards the gallbladder, the microorganisms might function as a trigger for the immune system and stimulate gallstone formation. Based on this study, four genera were potentially involved in cholesterol crystallization: Pseudomonas, Enterococcus, Klebsiella, and Enterobacter.

Furthermore, a recent study detected a higher prevalence of Bacteroidaceae, Prevotellaceae, Porphyromonadaceae, and Veillonellaceae in patients with gallstone disease [12] compared to individuals without diagnosed hepatobiliary pathology.

A different rate of cultured bacteria in the bile of patients with brown pigment lithiasis (between 53% and 100%), cholesterol gallstones (between 9% to 34%), and black pigment stones (from 9% to 19.6%) has been consistently reported [53–55]. A very recent study aimed to investigate the possible relation between the most abundant bile acids and the microbiota in gallstone disease [56]. A significant direct correlation between taurocholic acid (TCA) and taurochenodeoxycholic acid (TCDCA) and the bile microbiota alpha-diversity was reported. In more detail, the presence of TCA was associated with species, such as Jeotgalicoccus psychrophilus, Prevotella intermedia, and Haemophilus parainfluenzae in the bile, while TCDCA concentration showed a positive link with the presence of Microbacterium, Lutibacterium, and Sphingomonas genera and Prevotella intermedia species. In conclusion, the different abundance of TCDCA and TCA correlates with bile microbiota alpha-diversity and the appearance of specific opportunistic pathogens in the bile of patients affected by gallstone disease.

Despite the availability of promising studies in this setting, it is still unknown if microbiological markers for gallstone disease exist, making it an interesting field for future research, especially for patients suffering from recurrent lithiasis. Besides its role as a biomarker of biliary diseases, the ‘gut microbiota–bile acid–host’ system may also offer a target to be manipulated, either with the use of specific probiotic strains or dietary interventions and prebiotics, as a novel strategy to handle the diseases associated with defective bile acid metabolism, in the attempt to restore a beneficial diversity of bacteria.
Table 1. Gut and biliary microbiota in gallstone disease.

| Potential Pathogenesis Mechanism                                                                 | Phylum     | Family            | Genus          |
|-------------------------------------------------------------------------------------------------|------------|-------------------|----------------|
| Genetic/environmental factors, drugs, lifestyle, comorbidities                                | Lactobacillaceae |                   |                |
| Cholesterol/bilirubin hypersecretion                                                           | Clostridiaceae     | Clostridium        | Dorea          |
| Intestinal, metabolic or dietary factors                                                        | Acidaminococcaceae |                 | Veillonella    |
| Bacterial damaging effect                                                                      | Ruminococcaceae   |                   |                |
| Alteration of bile acids metabolism and host metabolic pathways                                | Lachnospiraceae   | Blautia            | Anaerostipes  |
| Release of proinflammatory and vasoactive substances                                           | Bacteroidetes     | Prevotella         | Bacteroides   |
| Role in energy intake, intestinal permeability, promotion of chronic pro-inflammatory states    | Fusobacteriaceae  | Fusobacterium      |                |
| Perturbation of gut microbiota:                                                                | Proteobacteria    |                   |                |
| alteration of bile acids metabolism and host metabolic pathways                                | Clostridiaceae    | Faecalibacterium  |                |
| Release of proinflammatory and vasoactive substances                                           | Eubacteriaceae    | Eubacterium        |                |
| Role in energy intake, intestinal permeability, promotion of chronic pro-inflammatory states    | Lachnospiraceae   | Lachnospira        | Roseburia      |
| ↓ Proteobacteria                                                                               | Desulfovibrionaceae | Desulfovibrio |                |
| ↓ Actinobacteria                                                                               | Bifidobacteriaceae | Bifidobacterium |                |
Biliary Microbiota In Gallstone Disease

Genetic/environmental factors, drugs, lifestyle, comorbidities

Cholesterol/bilirubin hypersecretion

Intestinal, metabolic or dietary factors

Bacterial damaging effect

Perturbation of gut microbiota:

- Alteration of bile acids metabolism and host metabolic pathways

- Release of proinflammatory and vasoactive substances

- Role in energy intake, intestinal permeability, promotion of chronic pro-inflammatory states

| Bacteroidetes | Prevotella |
|--------------|------------|
| ↓ Bacteroidetes | Rikenellaceae |
| | Paludibacteraceae |
| | Barnesiellaceae |
| | Muribaculaceae |
| ↑ Proteobacteria | Enterobacteriaceae |
| ↑ Firmicutes | Enterococcaceae |
| ↓ Bacteroidetes | Enterococcus |
| ↓ Synergistetes | Synergistaceae |
| | Pyramidobacter |
3. Microbiome and Biliary Cancer

The term “oncobiome” has been coined to describe the research field that studies how
the microbiome is involved in the development of neoplastic disease [57]. Oncobiome re-
search initially focused on colorectal cancer and in recent years has expanded into several
other malignancies [58]. The possible mechanisms by which the microbiome influences
cancer development include: (a) the role of bacterial toxins/metabolites on cancer onset
and development [59–61], (b) how the microbiome can modulate the host’s local and sys-
temic immune responses [62–64], and (c) specific changes in microbial and host metabo-
lism [65–67]. It has been hypothesized that these host–microbe interactions can occur at
both the local and systemic levels [68]. Herein, we provide an overview of the role of the
microbiome in the development and progression of biliary cancer.

Cancers of the biliary tract encompass those arising from the intrahepatic and extra-
hepatic bile ducts, the gallbladder, and the ampulla of Vater and represent the sixth most
common cause of malignant lesions in the gastrointestinal tract in western countries. We
summarized the possible gut and biliary microbiota components involved in these dis-
eases in Table 2.
Table 2. Summarizing the main microorganisms involved in cholangiocarcinoma and gallbladder cancer.

| Potential Pathogenesis Mechanism                                                                 | Phylum       | Family                  | Genus         |
|------------------------------------------------------------------------------------------------|--------------|-------------------------|---------------|
| Direct impact of bacterial toxins/metabolites on cancer initiation and growth                   | ↑ Firmicutes | Lactobacillaceae        | Lactobacillus |
| Modulation of the host local and systemic immune response                                      |              | Peptostreptococcaceae   |               |
|                                                                                               |              | Ruminococcaceae         |               |
| Alteration of microbial and host metabolism                                                     | ↑ Actinobacteria | Bifidobacteriaceae    | Actinomyces Alloscardovia |
| Interaction of gut microbiota on the bile acids metabolism pathways                             |              |                         |               |
| Direct impact of bacterial toxins/metabolites on cancer initiation and growth                   | ↑ Proteobacteria | Enterobacteriaceae    | Klebsiella    |
| Modulation of the host local and systemic immune response                                      |              | Methylophilaceae        |               |
|                                                                                               |              | Sino-bacteriaceae       |               |
|                                                                                               |              | Helicobacteracae        |               |
|                                                                                               |              | Erythrobacteraceae      | Novosphingobium |
| Alteration of microbial and host metabolism                                                     | ↑ Gemmatimonadetes |             |               |
| Interaction of gut microbiota on the bile acids metabolism pathways                             | ↑ Nitrospirae |                         |               |
|                                                                                               | ↑ Chloroflexi |                         |               |
|                                                                                               | ↑ Latescibacteria |                    |               |
|                                                                                               | ↑ Planctomycetes |                    |               |
|                                                                                               | ↑ Actinobacteria | Bifidobacteriaceae    | Actinomyces   |
| Biliary Microbiota in Gallbladder Cancer | Direct impact of bacterial toxins/metabolites on cancer initiation and growth | Modulation of the host local and systemic immune response | Alteration of microbial and host metabolism | Inflammation-induced carcinogenesis |
|----------------------------------------|---------------------------------------------------------------------------|---------------------------------------------------------|------------------------------------------|----------------------------------|
|                                          | ↑ Firmicutes                                                               |                                                         | ↑ Bacteroidetes                           | ↑ Fusobacteria                     |
|                                          | Enterococcaceae                                                           | Dialister                                               | Bacteroidaceae                           | Fusobacteriaceae                  |
|                                          | Streptococcaceae                                                          | Streptococcus                                           |                                          | Fusobacterium                     |
|                                          | Bacillaceae                                                               | Geobacillus                                             |                                          |                                  |
|                                          |                                                                            | Anoxybacillus                                           |                                          |                                  |
|                                          | ↑ Bacteroidetes                                                           | Bacteroidaceae                                          | Prevotella                               | Fusobacterium                     |
|                                          |                                                                            |                                                          | Bacteroides                              |                                  |
|                                          | ↑ Fusobacteria                                                            | Fusobacteriaceae                                        | Fusobacterium                           |                                  |
|                                          | ↑ Synergistetes                                                           | Synergistaceae                                          | Pyramidobacter                           |                                  |
|                                          | ↑ Deinococcus-Thermus                                                     | Thermaceae                                              | Meiothermus                              |                                  |
|                                          |                                                                            |                                                         |                                          |                                  |
3.1. Cholangiocarcinoma

Cholangiocarcinomas (CCA) are malignant neoplasms involving the intrahepatic or extrahepatic bile ducts and are usually classified, according to anatomy, into intrahepatic, perihilar, and distal cholangiocarcinomas. Given their high fatality rate and the silent progression of early disease, identifying the risk factors for the prevention and early detection of CCA is crucial. There are many known risk factors for the occurrence of CCA, including genetic background, chronic inflammation, environmental factors, and parasitic infections, such as Opisthorchis viverrini (OV) and Clonorchis sinensis. However, the exact mechanisms of carcinogenesis remain unclear. Experimental studies have shown an association between microbial gut dysbiosis and the development of CCA [69–71], probably related to the bile acid metabolism pathway.

It has been reported that conjugated bile acids promote tumorigenesis, whereas unconjugated bile acids inhibit CCA cell proliferation. Therefore, an increase in either secondary or conjugated bile acids could be protumorigenic [70]. In a recent study, elevated plasma/stool ratios of conjugated secondary bile acids (glycoursodeoxycholic acid and taoursodeoxycholic acid) were reported in CCA patients [72]. Moreover, in clinical studies conducted in the stools of CCA patients, a high richness (alpha-diversity) was reported compared to healthy individuals; in particular, a number of Lactobacillus, Actinomyces, Peptostreptococcaceae, Alloscardovia, and Bifidobacteriaceae were markedly increased. In addition, vascular invasion, which is a substantial prognostic factor, was associated with high levels of Ruminococcaceae species in the stools and higher circulating IL-4 levels [72]. Based on these results, it was speculated that the detection of plasma taoursodeoxycholic acid, together with the presence of Lactobacillus and Alloscardovia in the stools, could be used as a potential diagnostic noninvasive biomarker for CCA [72]. Despite these data highlighting the existence of a certain association between the gut microbiota, the bile acid profile, and CCA, future studies with rigorous designs and investigations of patient outcomes are needed [73].

Conversely, little is known about the biliary microbiome of CCA patients and its possible role in tumorigenesis. Indeed, while changes in the bile microbiome in patients with CCA have been reported, whether this a cause or an effect of the tumor development is unknown. The biliary microbiome is conventionally investigated on bile samples collected during ERCP. Increased levels of biliary Klebsiella pneumoniae were positively correlated with CCA [74]. Moreover, the presence of Helicobacter spp., including non-HP species, is more frequent in the bile of CCA patients than in those with benign biliary disorders. Moreover, the most virulent cagA-1 HP strain is more abundant in the bile of CCA patients compared with that of cholelithic or healthy individuals [75]. Based on these findings, it is possible to hypothesize a protumorigenic role for Helicobacter spp. through a pro-inflammatory cascade. Moreover, an assessment of the bile microbiota from ERCP samples by quantitative PCR showed an increase in species richness in the distal CCA patients compared with that of cholelithic patients. The most abundant species included Gemmatimonadetes, Nitrospirae, Chloroflexi, Latiscibacteria, and Planctomycetes [76]. As for culture-independent methods, Avilés-Jiménez and coworkers [71] published the first report investigating microbiota in the bile ducts of patients with cholangiocarcinoma by NGS. In this study, Methylphilaceae, Sinobacteriaceae, Actinomycetes, Dialister, Novosphingobium, Prevotella, Fusobacterium, and high-virulence HP were shown to be more abundant in the tumor tissue of CCA patients. A recent study investigated the microbiome of CCA and adjacent normal tissue and showed that Bifidobacteriaceae, Enterobacteriaceae, and Enterococcaceae were enriched in the tumor tissue specimens of CCA associated with liver fluke (O. viverrini) compared with CCA not associated with O. viverrini, suggesting a relationship between parasitic infections, the biliary microbiome, and the tissue microenvironment [77]. Saab et al. [78] recently analyzed the biliary microbiota of 28 CCA and 47 patients with gallstones, employed as control group. The most abundant genera in the CCA patients were Enterococcus, Streptococcus, Bacteroides, Klebsiella, and
Pyramidobacter. The relative abundance of genera, such as Bacteroides, Geobacillus, Meiothermus, and Anoxybacillus, was also associated with specific comorbidities.

In conclusion, these results support the view that changes in the gut, bile microbiota, and in the biliary epithelium may play a significant role in CCA pathogenesis. Despite the variation in species abundance, Helicobacter spp., Bifidobacteriaceae, and Ruminococcaceae are generally increased in CCA patients. However, the field of microbiome investigation in CCA is new, and both basic and clinical investigations are needed to confirm whether the biliary microbiome may be an attractive biomarker or therapeutic target.

3.2. Gallbladder Cancer

Gallbladder cancer (GBC) is a highly invasive neoplasm with a 5-year survival rate of less than 5% [79]. Little is known about its etiopathogenesis. However, various factors, such as genetic susceptibility, infections, and lifestyle have been associated with the occurrence of GBC. The association between chronic calculous cholecystitis (CCC) and GBC has been reported [80,81]. Persistent biliary bacterial infections may expose individuals to the risk of an environment conducive to the development of malignant neoplasia. Similar to the intestinal mechanisms, the biliary mucosa contains several barriers, such as chemical, mechanical, and immunological barriers, that are responsible for immunological tolerance against commensals. Proteobacteria, Firmicutes, and Bacteroidetes mainly populate the gallbladder ecosystem [10]. Moreover, chronic colonization of S. Typhi may be a primary predisposing factor for the onset of GBC [82]. Song et al. [83] performed a metagenomic sequencing of the mucosal microbiome of CCC and GBC patients, exploring specific changes during the development of the malignant neoplasia form of chronic cholecystitis. In this study, the core microbiota was similar in both groups, whereas, during the development of GBC, species richness and evenness decreased, with differences also seen in the biliary microbial composition. In particular, Peptostreptococcus stomatis, Fusobacterium mortiferum, and Enterococcus faecium were found to be positively correlated with GBC. Future large studies with genomic analyses are needed to explore the interactions between the immune system and the host microbiome in the growth of GBC.

4. Microbiome and Autoimmune and Cholestatic Liver Diseases

4.1. Microbiota and Primary Biliary Cholangitis

Primary biliary cholangitis (PBC) is an uncommon chronic inflammatory autoimmune cholestatic liver disease that can have a serious course and progress to end-stage liver disease when untreated [84]. Its pathogenesis is multifaceted and occurs through the interaction between the immune and biliary pathways, where genetic and environmental factors play a substantial role [85]. As it often occurs in autoimmune disorders, it has been suggested that molecular mimicry between host antigens and microbes may act as a possible trigger [86]. More recently, the study of the gut–liver axis and, in particular, the farnesoid X receptor (FXR)–fibroblast growth factor (FGF)-19 signaling pathway and its role in bile acid metabolism, has led to a better understanding of PBC pathogenesis and the development of new targets for PBC treatment.

Despite these presumptions, only a few studies to date have evaluated the composition of gut microbiota in PBC patients [87–90], and they are reported in Table 3. Tang et al. [88] demonstrated in their pivotal paper that PBC patients presented a decreased gut microbiota richness; at the phylum level, Bacteroides spp were significantly decreased and Fusobacteria and Proteobacteria spp were over-represented. The authors developed and validated a signature microbial signature based on 12 genera that could distinguish PBS patients from healthy controls with excellent accuracy; Enterabacteriacea, Klebsiella, Pseudomonas, Clostridium, Veillonella, Streptococcus, and Haemophilus were among the most relevant PBC-enriched genera. More recently, Chen and coworkers [91] elegantly captured the interplay between the bile acid profile, gut microbiota, and FGF-19 levels in PBC patients. Moreover, treatment with ursodeoxycholic acid (UDCA) reversed the abundance
of most of the PBC-associated genera [88,89] and, more interestingly, the microbiota profile could predict the UDCA response, as non-responders presented a significantly lower abundance of the genus Faecalibacterium, a known butyrate-producing beneficial bacteria [92].
Table 3. Main gut and biliary microbiota components involved in autoimmune liver disease.

| Potential Pathogenesis Mechanism | Phylum       | Family                | Genus          |
|----------------------------------|--------------|-----------------------|----------------|
| Interaction between immune and biliary pathways | ↑ Proteobacteria |                       |                |
| Genetic and environmental factors | ↑ Fusobacteria |                       |                |
| Molecular mimicry between host antigens and microbe | ↓ Bacteroidetes |                       |                |

| Gutt Microbiota in PBC                  |                       |                      |                |
|----------------------------------------|-----------------------|----------------------|----------------|
| Interaction between immune and biliary pathways |                       |                      |                |
| Genetic and environmental factors       | ↑ Firmicutes           | Staphylococcaceae    | Staphylococcus |
| Molecular mimicry between host antigens and microbe |       | Enterococcaceae      | Enterococcus   |

| Biliary Microbiota in PBC               |                       |                      |                |
|----------------------------------------|-----------------------|----------------------|----------------|
| Interaction between immune and biliary pathways |                       |                      |                |
| Genetic and environmental factors       | ↑ Firmicutes           | Staphylococcaceae    | Staphylococcus |
| Molecular mimicry between host antigens and microbe |       | Enterococcaceae      | Enterococcus   |

| Gutt Microbiota in PSC                  |                       |                      |                |
|----------------------------------------|-----------------------|----------------------|----------------|
| Gut dysbiosis                          | ↑ Firmicutes           | Enterococcaceae      | Enterococcus   |
| Alteration of intestinal permeability → bacterial translocation |       | Streptococcaceae     | Streptococcus  |
| Immune-mediated hepatobiliary inflammation |                       | Lactobacillaceae     | Lactobacillus  |
|                                        | ↑ Fusobacteria         | Fusobacteriaceae     | Fusobacterium  |
|                                        | ↑ Actinobacteria       | Micrococcaceae       | Rothia         |
|                                        | ↑ Bacteroidetes        | Tannerellaceae       | Parabacteroides|

| Biliary Microbiota in PSC               |                       |                      |                |
|----------------------------------------|-----------------------|----------------------|----------------|
| Gut dysbiosis                          | ↑ Firmicutes           | Staphylococcaceae    | Staphylococcus |
| Alteration of intestinal permeability → bacterial translocation |       | Enterococcaceae      | Enterococcus   |
|                                        | ↑ Bacteroidetes        | Bacteroidaceae       | Prevotella     |
| Gut Microbiota in AIH | Immune-mediated hepatobiliary inflammation |
|----------------------|------------------------------------------|
|                      | Increased intestinal permeability and gut microbiome dysbiosis |

| Bacterial Family | Subfamily | Genus |
|-----------------|-----------|-------|
| Fusobacteria    | Fusobacteriaceae | Fusobacterium |
| Proteobacteria  | Pasteurellaceae | Haemophilus |
| Firmicutes      | Acidaminococcaceae | Veillonella |
|                 | Lactobacillaceae | Lactobacillus |
|                 | Ruminococcaceae | Oscillospira |
To our knowledge, only one study has evaluated the microbiome of the biliary tract in PBC patients [93]. Hiramatsu et al. aseptically extracted the gallbladder bile in patients with PBC undergoing liver transplantation and controls (patients with primary sclerosing cholangitis (PSC), hepatitis C virus, cholecystitis, etc.) and detected bacterial species by PCR amplification in 10/15 patients with PBC; the most common were Gram-positive cocci, such as Staphylococcus aureus, Enterococcus faecium, Streptococcus pneumoniae or other streptococci, and Lactobacillus plantarum, and they differed significantly from controls, including patients with cholecystolithiasis.

In conclusion, PBC patients present a distinct microbial profile, both in the gastrointestinal and in the biliary tract. Whether this difference is a consequence of the altered bile acid composition in PBC patients or plays a pathogenetic role in the development and progression of the disease is still to be determined. Future studies should investigate whether microbiota composition could predict the response to UDCA treatment, the severity of the disease, and the development of liver-related events in patients with PBC.

### 4.2. Microbiota and Autoimmune Hepatitis

Autoimmune hepatitis (AIH) is a chronic, progressive, and immunologically mediated inflammatory liver disorder [92]. Recent studies in animal models have proposed that increased intestinal permeability and gut microbiome dysbiosis play a role in the pathogenesis of liver inflammation in AIH [93,94]. Table 3 reports some of the main bacteria involved in this setting. Lin et al. [95] described, for the first time in humans, the features of a leaky gut (disruption of the architecture of the duodenal mucosa and reduced expression of tight junction proteins, such as ZO-1 and occludin) and dysbiosis, with a significant decrease of *Bifidobacterium* and *Lactobacillus* in AIH patients as compared to healthy controls. Since then, several authors have evaluated the composition of gut microbiota in AIH patients [96–99]. Similar to what was previously reported for PBC patients, Wei et al. [96] identified and validated a gut microbiome signature of AIH, including four genera (*Veillonella*, *Lactobacillus*, *Oscillospira*, and *Clostridiales*) that could accurately distinguish AIH patients from healthy controls. Liwinski et al. [98] more recently showed that a disease-specific decline of the relative abundance of *Bifidobacterium* was observed in AIH patients, and this was associated with failure to achieve remission.

To date, no study has investigated the composition of the microbiome of the biliary tract in patients with AIH and its association with the histological and clinical features of this liver disease.

### 4.3. Microbiota and Primary Sclerosing Cholangitis

Primary sclerosing cholangitis (PSC) is another, even less common, chronic inflammatory disease of the liver and bile ducts that is associated with the development of cholangitis, progressive fibrosis, and end-stage disease requiring liver transplantation [100]. Unlike the above-mentioned diseases, no accepted medical therapy for PSC exists at the moment [101], and this is partly because the etiology and pathogenesis of PSC are not well-understood. As PSC can be associated with intestinal inflammatory liver disease (IBD), especially ulcerative colitis (UC), it has been long hypothesized that the interplay between the gut and hepatobiliary systems played a central role in PSC pathogenesis [102,103], mainly through gut dysbiosis, alteration of intestinal permeability, bacterial translocation, and immune-mediated hepatobiliary inflammation [104–106].

In this view, several studies have investigated the gut microbiota composition in PSC patients [107–117], as reported in Table 3. The diversity of PSC is significantly reduced in PSC patients, and its global composition is distinct from that of patients with UC or healthy controls [110,111]; on the other hand, whether there is a difference between PSC patients with and without IBD is controversial [110,113]. At a genus level, the microbiota of PSC patients was overrepresented in *Enterococcus* [111,113,115,116], *Lactobacillus* [111,115,116], *Veillonella* [110,113,114,116], *Streptococcus* [113,115,116], *Fusobacterium* [111],...
Rothia [113], and Parabacteroides [116]. In the largest cohort to date, including patients from a German and Norwegian cohort, Kummer et al. [109] not only identified species associated with PSC patients but also a decrease in the abundance of genes related to the synthesis of vitamin B6 and branched-chain amino acids, and this reduction was associated with lower liver-transplantation-free survival. Finally, Lemoinne et al. [114] showed that PSC patients also displayed a fungal gut dysbiosis, with an increased proportion of Exophiala and a decreased proportion of Saccharomyces.

As for the microbiota of the biliary tract, only a few studies have evaluated its composition by cultures or PCR amplification techniques [91, 118–122]. Olsson et al. [118, 119] first analyzed the microbiota composition of bile samples from explanted livers of 36 patients with PSC. The authors reported positive cultures in 20 (56%) patients, where Strep-tococci (16/20), Enterococci (5/20), and Staphylococci (5/20) were the most common bacteria; however, these results might have been biased by culture contamination, recent ERCP, and the use of antibiotics [119]. In the above-mentioned study by Hiramatsu et al. [91], bacterial cultures were positive in one out of five patients (Streptococcus milleri), and PCR amplification identified bacteria species in two patients (S. milleri and S. aureus). In the more recent study by Pereira et al. [121], including bile samples from 80 PSC patients and 46 controls undergoing ERCP, the most common genera in the bile tract were Prevotella, Streptococcus, Veillonella, Fusobacterium, and Haemophilus.

From a clinical point of view, given this substantial evidence linking gut microbiota and autoimmune cholangitis, different trials have investigated the therapeutic role of different antibiotics [101], such as metronidazole [123, 124], vancomycin [123–125], and rifaximin [126], with inconclusive results. Of note, in their pilot study, Allegretti et al. [127] showed that fecal microbiota transplantation (FMT) was safe in 10 patients with PSC and concomitant IBD; a decrease in the alkaline phosphatase levels ≥50% was seen in 3 (30%) patients, and this correlated with the bacterial diversity and engraftment after FTM.

In conclusion, PSC is a rare disease with a complex pathogenesis, where gut and biliary tract dysbiosis may play a significant role. The therapeutic role of microbiome modulation in these patients is an intriguing field of research that needs further investigation.

5. Microbiome and Liver Transplantation

Liver transplantation (LT) is associated with an overall medical and functional recovery for most patients with end-stage liver disease, and changes in gut microbial composition are no exception. We reported the main evidence on this topic in Table 4. Several studies [128–131] have shown that at 6 months after LT, the gut microbiota is significantly improved, with increased diversity, increased beneficial autochthonous taxa, such as Ruminococcaceae, Lachnospiraceae, and Akkermansia, and a reduction in the pathogenic genera belonging to Enterobacteriaceae (i.e., Escherichia, Shigella, and Salmonella). However, up to 30% of the patients showed persistent cognitive impairment, and this was associated with a relative abundance in Proteobacteria after LT.
Table 4. LT-related gut and bile microbiota alternations.

| Potential Pathogenesis Mechanism | Phylum       | Family               | Genus      |
|---------------------------------|--------------|----------------------|------------|
| **Gut Microbiota in Liver Transplant** | ↑ Firmicutes | Ruminococcaceae      | Enterococcus |
| Interaction between immunosuppression, antibiotic therapy, infections, and liver allograft immunity | Lachnospiraceae | Clostridiaceae       |            |
| Modulation of both innate and adaptive liver allograft immunity → prevention of complications such as ischemia-reperfusion injury, acute cellular rejection, and infections after LT. | Streptococcaceae | Enterococcaceae      |            |
|                                 | ↑ Verrucomicrobia | Verrucomicrobiaceae | Akkermansia |
|                                 | ↑ Proteobacteria | Enterobacteriaceae | Klebsiella |
|                                 | ↑ Bacteroidetes | Bacteroidaceae      | Bacteroides |
|                                 | ↑ Actinobacteria | Bifidobacteriaceae  |            |
|                                 | ↓ Firmicutes   | Enterococcaceae      | Faecalibacterium |
|                                 | Clostridiaceae | Lachnospiraceae      |            |
|                                 | ↓ Proteobacteria | Enterobacteriaceae | Escherichia |
|                                 | Bacteroidetes | Bacteroidaceae    | Shigella    |
|                                 | ↓ Bacteroidetes | Bacteroidaceae     | Salmonella  |

| **Biliary Microbiota in Liver Transplant** | ↑ Firmicutes | Enterococcaceae | Enterococcus |
| Interaction between immunosuppression, antibiotic therapy, infections, and liver allograft immunity | Streptococcaceae | Streptococcus |
| Modulation of both innate and adaptive liver allograft immunity → prevention of complications such as ischemia-reperfusion injury, acute cellular rejection, and infections after LT. | Clostridiaceae | Lactococcus |
|                                 | ↑ Firmicutes   | Staphylococcaceae | Staphylococcus |
|                                 | Clostridiaceae | Staphylococcus    | Clostridium   |
ischemia-reperfusion injury, acute cellular rejection, and infections after LT.

| ↑ Proteobacteria | Enterobacteriaceae | Escherichia, Klebsiella |
|------------------|--------------------|------------------------|
| Rhizobiaceae     | Rhizobium          |
| Xanthomonadaceae | Nevskia, Stenotrophomas |
| Pseudomonaceae   | Pseudomonas        |
| Aeromonadaceae   | Aeromonas          |

| ↑ Ascomycota | Saccharomycetaceae | Candida |

| ↓ Proteobacteria | Enterobacteriaceae | Escherichia, Shigella, Salmonella |

| ↓ Bacteroidetes | Bacteroidaceae | Bacteroides |

| ↓ Firmicutes | Clostridiaceae | Faecalibacterium, Lachnospira |
| Lachnospiraceae | | Lachnospira |
Recent developments have shown that the microbiota plays a significant role in the development of complications after LT, where a complex interaction between immunosuppression, antibiotic therapy, infections, and liver allograft immunity takes place [132]. Animal studies have shown that gut bacteria shed microbial-associated molecule patterns into the portal venous circulation, shaping the number, functional activity, and maturational status of liver Kupffer cells [133]. These findings suggest that the gut microbiome is an important modulator of both innate and adaptive liver allograft immunity, and, therefore, its modulation could play a role in the prevention of complications, such as ischemia-reperfusion injury (IRI), acute cellular rejection (ACR), and infections after LT. For instance, both animal [134,135] and human studies [136,137] have shown that probiotic supplementation might attenuate the IRI entity. In fact, cirrhotic patients on rifaximin before LT [136] or patients receiving multi-strain probiotics before LT, in a recent randomized controlled trial (RCT) [137], had a lower incidence of early allograft dysfunction (EAD); this is probably due to the modulation of the intestinal microbiota, the suppression of inflammatory cell activation in the graft, and, therefore, the attenuation of the hepatic IRI, the main driver of post-LT organ dysfunction. Regarding ACR, studies in animals [138,139] and humans [140] have established a role of gut dysbiosis in its development, with microbiome alterations found as early as one week after LT. ACR patients presented with a lower microbiome diversity; an increase in Bacteroides, Enterobacteriaceae, Streptococcaceae, and Bifidobacteriaceae; and a decrease in Enterococcaceae, Lactobacillaceae, Clostridiaceae, and Ruminococcaceae. However, a recent meta-analysis failed to show a significant reduction in ACR incidence after probiotic supplementation in LT recipients [141].

It is noteworthy that gut microbiota is a strong predictor of infection after LT, a major cause of morbidity and mortality in these patients [140]. In a recent study [131], a low pre-transplant alpha-diversity, as well as a post-LT abundance of Enterococcus and Klebsiella, and reductions in Bacteroides, Facalibacterium, and Lachnospira, were associated with colonization by multidrug-resistance bacteria, a new hallmark of gut dysbiosis. Therefore, the restoration of the intestinal microbiota–host homeostasis has been an attractive strategy to prevent infection after LT, with very promising results. A meta-analysis of four RCTs [141] found that the synergic use of prebiotics and probiotics significantly reduced the infection rates (7% vs. 35%) after LT; these results were later confirmed by a fifth RCT [137]. Moreover, gut dysbiosis was also associated with the development of non-anastomotic biliary strictures [142], hepatic generation after partial liver grafts [132], and the recurrence of liver disease [143].

Finally, only a handful of studies has evaluated the composition of the bile microbiome in LT recipients, exclusively in patients undergoing ERCP/percutaneous transhepatic cholangiography for biliary complications [144]. In a prospective cohort of 213 patients, the authors showed that the most common bacterial isolates were Enterococcus spp. (40%), Streptococci (20.5%), Staphylococcus spp. (12.8%), Escherichia coli (10%), and Klebsiella spp. (4%); moreover, Candida albicans was also found in 15.6% of patients. Importantly, colonization by enteric bacteria, despite successful endoscopic treatment, and fungibilia were associated with lower retransplantation-free survival. Later, the composition of the bile microbiome by was confirmed culture-based techniques by a smaller prospective study [144]. Only in the studies by Liu et al. [145,146] was the bile microbiota assessed by 16S rRNA gene sequencing. The authors found that the most common genera were Enterococcus, Rhizobium, Neuska, Lactococcus, Bacillus, Clostridium sensu strictu, Stenotrophomonas, Pseudomonas, Streptococcus, and Aeroma. Moreover, the bile microbiome composition differed significantly between patients with clinical symptoms or signs of biliary obstruction and controls, with an increase in Proteobacteria and a decrease in Firmicutes phyla in the case group. In conclusion, these data should be interpreted with caution, as contamination of bile sampling, the use of antibiotics, previous ERCP, etc., are all confounding factors that should be considered. However, the study of the bile microbiome is very promising for a better understanding of the pathogenesis of biliary and non-biliary complications after LT.
6. Conclusions and Future Perspectives

The biliary tract is an interesting system interposed between an aseptic system, the hepatocyte, and a rich set of microbes, our gut. The understanding of the interaction of commensal biliary microbes with the host in determining the state of health or disease of the hepatobiliary system is an interesting topic of research, with the aim to define the concept of “biliary dysbiosis”.

While there is some evidence for an association between specific bacterial signatures and conditions, such as biliary stones, inflammatory disorders, or malignancies, causality is far from proven. In this view, therapies modulating the microbiota, with antibiotics, prebiotics, probiotics, and even FMT are intriguing possibilities to be tested in large prospective randomized studies for the management of these diseases.

Author Contributions: Conceptualization, writing, review and editing, C.B., G.G., C.C., M.S., E.D. and E.S. Supervision and final editing, G.C., V.S., A.C., G.E. and C.F. All authors have read and agreed to the published version of the manuscript.

Funding: No grants or other financial support.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare that they have no conflict of interest.

References

1. Sinagra, E.; Utzeri, E.; Morreale, G.C.; Fabbri, C.; Pace, F.; Anderloni, A. Microbiota-gut-brain axis and its affect in inflammatory bowel disease: Pathophysiologocal concepts and insights for clinicians. World J. Clin. Cases 2020, 8, 1013–1025.
2. Marchesi, J.R.; Ravel, J. The vocabulary of microbiome research: A proposal. Microbiome 2015, 3, 31. https://doi.org/10.1186/s40168-015-0094-5.
3. Dupre, J.; O’Malley, M.A. Varieties of Living Things: Life at the Intersection of Lineage and Metabolism. Philos. Theory Biol. 2009, 1. https://doi.org/10.3998/ptb.6959004.0001.003.
4. Whipp, J.M.; Lewis, K.; Cooke, R.C. Mycoparasitism and plant disease control 161–187. In Fungi in Biological Control Systems; Burge, N.M.; Ed.; Manchester University Press: Manchester, UK, 1988; p. 176.
5. Berg, G.; Rybakova, D.; Fischer, D.; Cernava, T.; Vergès, M.C.C.; Charles, T.; Chen, X.; Cocolin, L.; Eversole, K.; Corral, G.H.; et al. Microbiome definition re-visited: Old concepts and new challenges. Microbiome 2020, 8, 103.
6. Cresci, G.A.; Bawden, E. Gut microbiome: What we do and don’t know. Nutr. Clin. Pract. 2015, 30, 734–746.
7. Do Nascimento, F.S.; Suzuki, M.O.; Tabá, J.V.; De Mattos, V.C.; Pipek, L.Z.; D’Albuquerque, E.M.C.; Iuamoto, L.; Meyer, A.; Andraus, W.; Pinho, J.R.R.; et al. Analysis of biliary MICROBiota in hepatobiliary pancreatic diseases compared to healthy people [MICROBILIO]: Study protocol. PLoS ONE 2020, 15, e0242553. https://doi.org/10.1371/journal.pone.0242553.
8. Jandhyala, S.M.; Talukdar, R.; Subramanyam, C.; Vuyyuru, H.; Sasikala, M.; Reddy, D.N. Role of the normal gut microbiota. World J. Gastroenterol. 2015, 21, 8787–8803. https://doi.org/10.3748/wjg.v21.i29.8787.
9. Kostic, A.D.; Xavier, R.J.; Gevers, D. The Microbiome in Inflammatory Bowel Disease: Current Status and the Future Ahead. Gastroenterology 2014, 146, 1489–1499. https://doi.org/10.1053/j.gastro.2014.02.009.
10. Jiménez, E.; Sánchez, B.; Farina, A.; Margolles, A.; Rodríguez, J.M. Characterization of the bile and gall bladder microbiota of healthy pigs. Microbiol. Open 2014, 3, 937–949. https://doi.org/10.1002/mbo3.218.
11. Nicoletti, A.; Ponziani, F.R.; Nardella, E.; Ianoro, G.; Gasbarrini, A.; Dal Verme, L.Z. Biliary tract microbiota: A new kid on the block of liver diseases? Eur. Rev. Med. Pharmacol. Sci. 2020, 24, 2750–2775.
12. Molinero, N.; Ruiz, L.; Milani, C.; Gutiérrez-Díaz, I.; Sánchez, B.; Mangifesta, M.; Segura, J.; Cambero, I.; Campelo, A.B.; García-Bernardo, C.M.; et al. The human gallbladder microbiome is related to the physiological state and the biliary metabolic profile. Microbiome 2019, 7, 100. https://doi.org/10.1186/s40168-019-0712-8.
13. Wang, Y.; Qi, M.; Qin, C.; Hong, J. Role of the biliary microbiome in gallstone disease. Expert Rev. Gastroenterol. Hepatol. 2018, 12, 1193–1205. https://doi.org/10.1080/17474142.2018.1533812.
14. Lammert, F.; Acalovschi, M.; Ercolani, G.; van Erpecum, K.J.; Gurusamy, K.S.; van Laarhoven, C.J.; Portincasa, P. EASL Clinical Practice Guidelines on the prevention, diagnosis and treatment of gallstones. J. Hepatol. 2016, 65, 146–181. https://doi.org/10.1016/j.jhep.2016.03.005.
15. Lammert, F.; Gurusamy, K.; Ko, C.W.; Miquel, J.-F.; Méndez-Sánchez, N.; Portincasa, P.; van Erpecum, K.J.; van Laarhoven, C.J.; Wang, D.Q.-H. Gallstones. Nat. Rev. Dis. Prim. 2016, 2, 16024–16024. https://doi.org/10.1038/nrdp.2016.24.
16. Portincasa, P.; Moschetta, A.; Palasciano, G. Cholesterol gallstone disease. *Lancet* 2006, 368, 230–239. https://doi.org/10.1016/s0140-6736(06)6844-2.

17. Marshall, H.U.; Einarsson, C. Gallstone disease. *J. Intern. Med.* 2007, 261, 529–542.

18. Gutiérrez-Díaz, I.; Molinero, N.; Cabrera, A.; Rodríguez, J.I.; Margolles, A.; Delgado, S.; González, S. Diet: Cause or Consequence of the Microbial Profile of Cholelithiasis Disease? *Nutrients* 2018, 10, 1307. https://doi.org/10.3390/nu10091307.

19. Cai, J.-S.; Chen, J.-H. The Mechanism of Enterohepatic Circulation in the Formation of Gallstone Disease. *J. Membr. Biol.* 2014, 247, 1067–1082. https://doi.org/10.1007/s00232-014-9715-3.

20. Ramírez-Pérez, O.; Cruz-Ramón, V.; Chinchilla-López, P.; Méndez-Sánchez, N. The Role of the Gut Microbiota in Bile Acid Metabolism. *Ann. Hepatol.* 2017, 16, s15–s20.

21. Wu, T.; Zhang, Z.; Liu, B.; Hou, D.; Liang, Y.; Zhang, J.; Shi, P. Gut microbiota dysbiosis and bacterial community assembly associated with cholesterol gallstones in large-scale study. *BMC Genom.* 2013, 14, 669–669. https://doi.org/10.1186/1471-2164-14-669.

22. Wang, H.H.; Portincasa, P.; Afdhal, N.H.; Wang, D.Q.H. Leth genes and genetic analysis of cholesterol gallstone for-mation. *Gastroenterol. Clin. N. Am.* 2010, 39, 185–207.

23. Fremont-Rahl, J.J.; Ge, Z.; Umama, C.; Whary, M.T.; Taylor, N.S.; Muthupalani, S.; Carey, M.C.; Fox, J.G.; Maurer, K.J. An Analysis of the Role of the Indigenous Microbiota in Cholesterol Gallstone Pathogenesis. *PLoS ONE* 2013, 8, e70657. https://doi.org/10.1371/journal.pone.0070657.

24. Maki, T. Pathogenesis of calcium bilirubinate gallstone: Role of *E. coli*, beta-glucuronidase and coagulation by inorganic ions, polyelectrolytes and agitation. *Ann. Surg.* 1966, 164, 90–100. https://doi.org/10.1097/00000658-196607000-00010.

25. Maluenda, F.; Csendes, A.; Burdiles, P.; Diaz, J. Bacteriological study of choledochal bile in patients with common bile duct stones, with or without acute suppurrative cholangitis. *Hepatogastroenterology* 1989, 36, 132–135.

26. Kaufman, H.S.; Magnuson, T.H.; Lillemeoe, K.D.; Frasca, P.; Pitt, H.A. The Role of Bacteria in Gallbladder and Common Duct Stone Formation. *Ann. Surg.* 1989, 209, 584–592. https://doi.org/10.1097/00000658-198905000-00011.

27. Nakano, T.; Yanagisawa, J.; Nakayama, F. Phospholipase activity in human bile. *Hepatology* 1988, 8, 1560–1564. https://doi.org/10.1002/hep.1840080615.

28. Gumucio, J.J.; Ostrow, J.D. Brown pigment gallstones: The role of bacterial hydrolyses and another missed opportunity. *Hepatology* 1991, 13, 607–609. https://doi.org/10.1002/hep.18410335.

29. Lee, D.K.; Tarr, P.J.; Haigh, G.W.; Lee, S.P. Bacterial Dra in Mixed Cholesterol Gallstones. *Am. J. Gastroenterol.* 1999, 94, 3502–3506. https://doi.org/10.1111/j.1572-0241.1999.01614.x.

30. Kawai, M.; Iwahashi, M.; Uchiyama, K.; Ochiai, M.; Tanimura, H.; Yamaue, H. Gram-positive cocci are associated with the formation of completely pure cholesterol stones. *Am. J. Gastroenterol.* 2002, 97, 83–88. https://doi.org/10.1111/j.1572-0241.2002.05425.x.

31. Yoon, W.J.; Kim, H.-N.; Park, E.; Ryu, S.; Chang, Y.; Shin, H.; Kim, H.-L.; Yi, S. The Impact of Cholecystectomy on the Gut Microbiota: A Case-Control Study. *J. Clin. Med.* 2019, 8, 79. https://doi.org/10.3390/jcm8010079.

32. Zhu, L.; Zhang, H.; Li, J.; Wang, X.Z.; Cai, D.; Zhang, Y.L. Study on the mechanism of pronucleation of cholesterol crystal formation by bacteria. *J. Hepatopancreatobiliary Surg.* 2009, 21, 122–125.

33. Keren, N.; Konikoff, F.M.; Paitan, Y.; Gabay, G.; Reshef, L.; Naftail, T.; Gophna, U. Interactions between the intestinal microbiota and bile acids in gallstones patients. *Environ. Microbiol. Rep.* 2015, 7, 874–880. https://doi.org/10.1111/1758-2229.12319.

34. Farina, A.; Dumouneac, J.; Lescury, P. Proteomic analysis of human bile and potential applications for cancer diagnosis. *Expert Rev. Proteomics* 2009, 6, 285–301.

35. Chang, C.-M.; Chiu, T.H.T.; Chang, C.-C.; Lin, M.-N.; Lin, C.-L. Plant-Based Diet, Cholesterol, and Risk of Gallstone Disease: A Prospective Study. *Nutrients* 2019, 11, 335. https://doi.org/10.3390/n11020335.

36. Chen, M.L.; Takeda, K.; Sundrud, M.S. Emerging roles of bile acids in mucosal immunity and inflammation. *Mucosal. Immunol.* 2019, 12, 851–861. https://doi.org/10.1038/s41385-019-0162-4.

37. Csendes, A.; Burdiles, P.; Maluenda, F.; Diaz, J.C.; Csendes, P.; Mitru, N. Simultaneous Bacteriologic Assessment of Bile from Gallbladder and Common Bile Duct in Control Subjects and Patients with Gallstones and Common Duct Stones. *Arch. Surg.* 1996, 131, 389–394. https://doi.org/10.1001/archsurg.1996.01430160047008.

38. Helaly, G.F.; El-Ghazawzy, E.F.; Kazeiw, A.H.; Dowidar, N.L.; Anwar, M.M.; Attia, N.M. Detection of Helicobacter Pylori Infection in Egyptian patients with chronic calcular cholecystitis. *Br. J. Biomed. Sci.* 2014, 71, 13–18. https://doi.org/10.1080/09674845.2014.11669957.

39. Neri, V.; Margiotta, M.; De Francesco, V.; Ambrosi, A.; Della Valle, N.; Fersini, A.; Tartaglia, N.; Minenna, M.F.; Ricciardelli, C.; Giorgio, F.; et al. DNA sequences and proteic antigens of *H. pylori* in cholecytic bile and tissue of patients with gallstones. *Aliment. Pharmacol. Ther.* 2005, 22, 715–720. https://doi.org/10.1111/j.1365-2036.2005.02644.x.

40. Swidsinski, A.; Ludwig, W.; Pahlig, H.; Priem, F. Molecular genetic evidence of bacterial colonization of cholesterol gallstones. *Gastroenterology* 1995, 108, 860–864. https://doi.org/10.1016/0016-5085(94)01215-2.

41. Ye, F.; Shen, H.; Li, Z.; Meng, F.; Li, L.; Yang, J.; Chen, Y.; Bo, X.; Zhang, X.; Ni, M. Influence of the Biliary System on Biliary Bacteria Revealed by Bacterial Communities of the Human Biliary and Upper Digestive Tracts. *PLoS ONE* 2016, 11, e0150519. https://doi.org/10.1371/journal.pone.0150519.
42. Peng, Y.; Yang, Y.; Liu, Y.; Nie, Y.; Xu, P.; Xia, B.; Tian, F.; Sun, Q. Cholesterol gallstones and bile host diverse bacterial communities with potential to promote the formation of gallstones. *Microb. Pathog.* 2015, 83-84, 57–63. https://doi.org/10.1016/j.micpath.2015.05.002.

43. Marshall, J.M.; Flechtner, A.D.; La Perle, K.M.; Gunn, J.S. Visualization of Extracellular Matrix Components within Sectioned Salmonella Biofilms on the Surface of Human Gallstones. *PLoS ONE* 2014, 9, e89243. https://doi.org/10.1371/journal.pone.0089243.

44. Belzer, C.; Kusters, J.G.; Kuipers, E.J.; Van Vliet, A.H.M. Urease induced calcium precipitation by Helicobacter species may initiate gallstone formation. *Gut* 2006, 55, 1678–1679. https://doi.org/10.1136/gut.2006.098319.

45. Kasprzak, A.; Szymt, M.; Malkowski, W.; Przybyszewska, W.; Helak-Lapaj, C.; Seraszek-Jaros, A.; Surdacka, A.; Malkowska-Lanzafame, A.; Kaczmarek, E. Analysis of immunohistochemical expression of proinflammatory cytokines (IL-17, IL-6, and TNF-7) in gallbladder mucosa: Comparative study in acute and chronic calculous cholecystitis. *Folia Morphol.* 2015, 74, 65–72. https://doi.org/10.5603/FM.2015.0011.

46. Sipos, P.; Krisztina, H.; Blázovics, A.; Fehér, J. Cholecystitis, gallstones and free radical reactions in human gallbladder. *Med. Sci. Monit.* 2001, 7, 84–88.

47. Moliner, N.; Ruiz, L.; Sánchez, B.; Argolles, A.; Delgado, S. Intestinal Bacteria Interplay With Bile and Cholesterol Metabolism: Implications on Host Physiology. *Front. Physiol.* 2019, 10, 185. https://doi.org/10.3389/fphys.2019.00185.

48. Kose, S.H.; Grice, K.; Orsi, W.D.; Ballal, M.; Coolen, M.J.L. Metagenomics of pigmented and cholesterol gallstones: The putative role of bacteria. *Sci. Rep.* 2018, 8, 1–13. https://doi.org/10.1038/s41598-018-29571-8.

49. Shen, H.; Ye, F.; Xie, L.; Yang, J.; Li, Z.; Xu, P.; Meng, F.; Li, L.; Xiaochen, B.; Bo, X.; et al. Metagenomic sequencing of bile from gallstone patients to identify different bacterial community patterns and novel biliary bacteria. *Sci. Rep.* 2015, 5, 17450. https://doi.org/10.1038/srep17450.

50. Segata, N.; Boernigen, D.; Tickle, T.L.; Morgan, X.C.; Garrett, W.S.; Huttenhower, C. Computational met‘omics for microbial community studies. *Mol. Syst. Biol.* 2013, 9, 666. https://doi.org/10.1038/msb.2013.22.

51. Natsui, M.; Honma, T.; Genda, T.; Nakadaira, H. Effects of endoscopic papillary balloon dilation and endoscopic sphincterotomy on bacterial contamination of the biliary tract. *Eur. J. Gastroenterol. Hepatol.* 2011, 23, 818–824. https://doi.org/10.1097/meg.0b013e328348c0bf.

52. Su, Y.; Wu, S.-D.; Jin, J.; Zhang, Z.-H.; Fan, Y. Role of intestinal barrier in pathogenesis of pigment gallstone in a guinea pig model. *Hepatobiliary Pancreat. Dis. Int.* 2006, 5, 443–448.

53. Cetta, F. The Role of Bacteria in Pigment Gallstone Disease. *Ann. Surg.* 1991, 213, 315–326. https://doi.org/10.1097/00000658-199104000-00006.

54. Stewart, L.; Griffiss, J.M.L.; Way, L.W. Spectrum of gallstone disease in the veterans population. *Am. J. Surg.* 2005, 190, 746–751. https://doi.org/10.1016/j.amjsurg.2005.07.014.

55. Abeysuriya, V.; Deen, K.; Wijesuriya, T.; et al. Microbiology of gallbladder bile in uncomplicated symptomatic chole-lithiasis-Related Complications. *Hepatobiliary Pancreat. Dis. Int.* 2008, 7, 633–637.

56. Petrov, V.A.; Fernández-Peralbo, M.A.; Derks, R.; Knyazeva, E.M.; Merzlikin, N.V.; Sazonov, A.E.; Mayboroda, O.A.; Saltykova, I.V. Biliary Microbiota and Bile Acid Composition in Cholelithiasis. *BioMed Res. Int.* 2020, 2020, 1–8. https://doi.org/10.1155/2020/1242364.

57. Thomas, R.M.; Jobin, C. The Microbiome and Cancer: Is the “Oncobiome” Mirage Real? *Trends Cancer* 2015, 1, 24–35.

58. Helmink, B.A.; Khan, M.A.W.; Hermann, A.; Gopalakrishnan, V.; Wargo, J.A. The microbiome, cancer, and cancer therapy. *Nat. Med.* 2019, 25, 377–388.

59. Arthur, J.C.; Perez-Chanona, E.; Mühlbauer, M.; Tomokvich, S.; Uronis, J.M.; Tan, T.-J.; Campbell, B.J.; Abujameel, T.; Dogan, B.; Rogers, A.B.; et al. Intestinal Inflammation Targets Cancer-Inducing Activity of the Microbiota. *Science* 2012, 338, 120–123. https://doi.org/10.1126/science.1224820.

60. Graillot, V.; Dormoy, I.; Dupuy, J.; Shay, J.W.; Huc, L.; Mirey, G.; Vignard, J. Genotoxicity of Cytotoxic Distending Toxin (CDT) on Isogenic Human Colorectal Cell Lines: Potential Promoting Effects for Colorectal Carcinogenesis. *Front. Cell. Infect. Microbiol.* 2016, 6, 34. https://doi.org/10.3389/fcimb.2016.00034.

61. Hong, X.; Tang, B.; Li, B.-S.; Xie, R.; Hu, C.-J.; Luo, G.; Qin, Y.; Dong, H.; Yang, S.-M. Helicobacter pylori virulence factor CagA promotes tumorigenesis of gastric cancer via multiple signaling pathways. *Cell Commun. Signal.* 2015, 13, 1–13. https://doi.org/10.1186/s12964-015-0111-0.

62. Li, R.; Zhou, R.; Wang, H.; Li, W.; Pan, M.; Yao, X.; Zhan, W.; Yang, S.; Xu, L.; Ding, Y.; et al. Gut microbiota-stimulated cathespin K secretion mediates TLR4-dependent M2 macrophage polarization and promotes tumor metastasis in colorectal cancer. *Cell Death Differ.* 2019, 26, 2447–2463. https://doi.org/10.1038/s41418-019-0312-y.

63. Calcinoatto, A.; Brevi, A.; Chesi, M.; Ferrarese, R.; Perez, L.G.; Grioni, M.; Kumar, S.; Garbitt, V.M.; Shari, M.E.; Henderson, K.J.; et al. Microbiota-driven interleukin-17-producing cells and eosinophils synergize to accelerate multiple myeloma progression. *Nat. Commun.* 2018, 9, 1–13. https://doi.org/10.1038/s41467-018-07305-8.

64. Yu, A.I.; Zhao, L.; Eaton, K.A.; Ho, S.; Chen, J.; Poe, S.; Becker, J.; Gonzalez, A.; McKinstry, D.; Hasso, M.; et al. Gut Microbiota Modulate CD8 T Cell Responses to Influence Colitis-Associated Tumorigenesis. *Cell Rep.* 2020, 31, 107471. https://doi.org/10.1016/j.celrep.2020.03.035.
65. Ma, C.; Han, M.; Heinrich, B.; Fu, Q.; Zhang, Q.; Sandhu, M.; Agdashian, D.; Terabe, M.; Berzofsky, J.A.; Fako, V.; et al. Gut microbiome-mediated bile acid metabolism regulates liver cancer via NKT cells. Science 2018, 360, eaan5931. https://doi.org/10.1126/science.aan5931.

66. Cohen, L.J.; Estehazy, D.; Kim, S.-H.; Lemetre, C.; Aguilar, R.R.; Gordon, E.A.; Pickard, A.J.; Cross, J.R.; Emiliano, A.B.; Han, S.M.; et al. Commensal bacteria make GPCR ligands that mimic human signalling molecules. Nature 2017, 549, 48–53. https://doi.org/10.1038/nature23874.

67. Bishehsari, F.; Engen, P.A.; Preite, N.Z.; Tuncil, Y.E.; Naqib, A.; Shaikh, M.; Rossi, M.; Wilber, S.; Green, S.J.; Hamaker, B.R.; et al. Dietary Fiber Treatment Corrects the Composition of Gut Microbiota, Promotes SCFA Production, and Suppresses Colon Carcinogenesis. Genes 2018, 9, 102. https://doi.org/10.3390genes9020102.

68. Schwabe, R.F.; Jobin, C. The microbiome and cancer. Nat. Rev. Cancer 2013, 13, 800–812.

69. Liu, R.; Zhao, R.; Zhou, X.; Liang, X.; Campbell, D.J.W.; Zhang, X.; Zhang, L.; Shi, R.; Wang, G.; Pandak, W.M.; et al. Conjugated bile acids promote cholangiocarcinoma cell invasive growth through activation of sphingosine 1-phosphate receptor 2. Hepatology 2014, 60, 908–918. https://doi.org/10.1002/hep.27085.

70. Dai, J.; Wang, H.; Dong, Y.; Zhang, Y.; Wang, J. Bile Acids Affect the Growth of Human Cholangiocarcinoma via NF-κB Pathway. Cancer Investigat. 2013, 31, 111–120. https://doi.org/10.3109/07357907.2012.762781.

71. Axeléz-Jiménez, F.; Guitron, A.; Segura-López, F.; Méndez-Tenorio, A.; Iwai, S.; Hernández-Guerrero, A.; Torres, J. Microbiota studies in the bile duct strongly suggest a role for Helicobacter pylori in extrahepatic cholangiocarcinoma. Clin. Microbiol. Infect. 2016, 22, 178.e11–178.e22. https://doi.org/10.1016/j.cmi.2015.10.008.

72. Jia, X.; Lu, S.; Zeng, Z.; Liu, Q.; Dong, Z.; Chen, Y.; Zhu, Z.; Hong, Z.; Zhang, T.; Du, G.; et al. Characterization of Gut Microbiota, Bile Acid Metabolism, and Cytokines in Intrahepatic Cholangiocarcinoma. Hepatology 2020, 71, 893–906. https://doi.org/10.1002/hep.30882.

73. Herraez, E.; Romero, M.R.; Macias, R.I.R.; Monte, M.J.; Marin, J.I.G. Clinical relevance of the relationship between changes in gut microbiota and bile acid metabolism in patients with intrahepatic cholangiocarcinoma. Hepatobiliary Surg. Nutr. 2020, 9, 211–214. https://doi.org/10.21037/hbsn.2019.10.11.

74. Di Carlo, P.; Serra, N.; D’Arpa, F.; Agrusa, A.; Gulotta, G.; Fasciana, T.; Rodolico, V.; Giammanco, A.; Sergi, C. The microbiota of the bilio-pancreatic system: A cohort, STROBE-compliant study. Infect. Drug Resist. 2019, 12, 1513–1527. https://doi.org/10.2147/ird.s200378.

75. Boonyanugomol, W.; Chomvarin, C.; Sripa, B.; Bhudhisawasdi, V.; Khuntikeo, N.; Hahnvajanawong, C.; Chamsuwan, A. Helicobacter pylori in Thai patients with cholangiocarcinoma and its association with biliary inflammation and proliferation. HPB 2012, 14, 177–184. https://doi.org/10.1111/j.1477-2578.2011.00423.x.

76. Chen, B.; Fu, S.W.; Lu, L.; Zhao, H. A Preliminary Study of Biliary Microbiota in Patients with Bile Duct Stones or Distal Cholangiocarcinoma. BioMed Res. Int. 2019, 2019, 1–12. https://doi.org/10.1155/2019/1092563.

77. Chng, K.R.; Chan, S.H.; Ng, A.H.Q.; Li, C.; Jusakul, A.; Bertrand, D.; Wilm, A.; Choo, S.P.; Tan, D.M.Y.; Lim, K.H.; et al. Tissue Microbiome Profiling Identifies an Enrichment of Specific Enteric Bacteria in Opioidergic viverrini Associated Cholangiocarcinoma. eBioMedicine 2016, 8, 195–202. https://doi.org/10.1016/j.ebiom.2016.04.034.

78. Saab, M.; Mestivier, D.; Sohrabi, M.; Rodriguez, C.; Khonsari, M.R.; Faraji, A.; Sobhani, I. Characterization of biliary microbiota dysbiosis in extrahepatic cholangiocarcinoma. PLoS ONE 2021, 16, e0247798. https://doi.org/10.1371/journal.pone.0247798.

79. Li, M.; Zhang, Z.; Li, X.; Ye, J.; Wu, X.; Tan, Z.; Liu, C.; Shen, B.; Wang, X.-A.; Wu, W.; et al. Whole-exome and targeted gene sequencing of gallbladder carcinoma identifies recurrent mutations in the Erbb pathway. Nat. Genet. 2014, 46, 872–876. https://doi.org/10.1038/ng.3030.

80. Hsing, A.W.; Bai, Y.; Andreotti, G.; Rashid, A.; Deng, J.; Chen, J.; Goldstein, A.M.; Han, T.-Q.; Shen, M.C.; Fraumeni, J.F.; et al. Family history of gallstones and the risk of biliary tract cancer and gallstones: A population-based study in Shanghai, China. Int. J. Cancer 2007, 121, 832–838. https://doi.org/10.1002/ijc.22276.

81. Shrikhande, S.V.; Barreto, S.G.; Singh, S.; Udwadia, T.E.; Agarwal, A.K. Cholecystitis in gallbladder cancer: Coincidence, co-factor, or cause? Eur. J. Oncol. 2010, 36, 514–519.

82. Di Domenico, E.G.; Cavollo, I.; Pontone, M.; Toma, L.; Ensoli, F. Biofilm producing Salmonella typhi: Chronic coloni-ization and development of gallbladder cancer. Int. J. Mol. Sci. 2017, 18, 1887.

83. Song, X.; Wang, X.; Hu, Y.; Li, H.; Ren, T.; Li, Y.; Liu, L.; Li, L.; Li, X.; Wang, Z.; et al. A metagenomic study of biliary microbiome change along the cholecystitis-carcinoma sequence. Clin. Transl. Med. 2020, 10, e97. https://doi.org/10.1002/ctm2.97.

84. Hirschfeld, G.M.; Beuers, U.; Corpechot, C.; Invernezzi, P.; Jones, D.; Marzioni, M.; Schramm, C. EASL Clinical Practice Guidelines: The diagnosis and management of patients with primary biliary cholangitis. J. Hepatol. 2017, 67, 145–172. https://doi.org/10.1016/j.jhep.2017.03.022.

85. Hirschfeld, G.M.; Dyson, J.K.; Alexander, G.J.M.; Chapman, M.H.; Collier, J.; Hübischer, S.; Patanwala, I.; Pereira, S.P.; Thain, C.; Thorburn, D.; et al. The British Society of Gastroenterology/UK-PBC primary biliary cholangitis treatment and management guidelines. Gut 2018, 67, 1568–1594. https://doi.org/10.1136/gutjnl-2017-315259.

86. Quigley, E.M.M. Primary Biliary Cirrhosis and the Microbiome. Semin. Liver Dis. 2016, 36, 349–353. https://doi.org/10.1055/s-0036-1594006.

87. Lv, L.-X.; Fang, D.-Q.; Shi, D.; Chen, D.-Y.; Yan, R.; Zhu, Y.-X.; Chen, Y.-F.; Shao, L.; Guo, F.-F.; Wu, W.-R.; et al. Alterations and correlations of the gut microbiome, metabolism and immunity in patients with primary biliary cirrhosis. Environ. Microbiol. 2016, 18, 2272–2286. https://doi.org/10.1111/1462-2920.13401.
88. Tang, R.; Wei, Y.; Li, Y.; Chen, W.; Chen, H.; Wang, Q.; Yang, F.; Miao, Q.; Xiao, X.; Zhang, H.; et al. Gut microbial profile is altered in primary biliary cholangitis and partially restored after UDCA therapy. Gut 2018, 67, 534–541. https://doi.org/10.1136/gutjnl-2016-313332.

89. Chen, W.; Wei, Y.; Xiong, A.; Li, Y.; Guan, H.; Wang, Q.; Miao, Q.; Bian, Z.; Xiao, X.; Lian, M.; et al. Comprehensive Analysis of Serum and Fecal Bile Acid Profiles and Interaction with Gut Microbiota in Primary Biliary Cholangitis. Clin. Rev. Allergy Immunol. 2020, 58, 25–38. https://doi.org/10.1007/s12016-019-08731-2.

90. Furukawa, M.; Moriya, K.; Nakayama, J.; Inoue, T.; Momoda, R.; Kawaratahi, H.; Namisaki, T.; Sato, S.; Douhara, A.; Kaji, K.; et al. Gut dysbiosis associated with clinical prognosis of patients with primary biliary cirrhosis. Hepatol. Res. 2020, 50, 840–852. https://doi.org/10.1111/hepr.13509.

91. Hiramatani, K.; Harada, K.; Tsuneyama, K.; Sasaki, M.; Fujita, S.; Hashimoto; Tan; Kaneko, S.; Kobayashi, K.; Nakamura, Y. Amplification and sequence analysis of partial bacterial 16S ribosomal RNA gene in gallbladder bile from patients with primary biliary cirrhosis. J. Hepatol. 2000, 33, 9–18. https://doi.org/10.1016/s0168-8278(00)0153-1.

92. Lohse, A.W.; Chazouilleres, O.; Daledos, G.; Drenth, J.; Heneghan, M.; Hofer, H.; Lammert, F.; Lenz, M. EASL clinical practice guidelines: Autoimmune hepatitis. J. Hepatol. 2015, 63, 971–1004. https://doi.org/10.1016/j.jhep.2015.06.030.

93. Yuksel, M.; Wang, Y.; Tai, N.; Peng, J.; Guo, J.; Beland, K.; Lapiere, P.; David, C.; Alvarez, F.; Colle, I.; et al. A novel “humanized mouse” model for autoimmune hepatitis and the association of gut microbiota with liver inflammation. Hepatology 2015, 62, 1536–1550. https://doi.org/10.1002/hep.27998.

94. Cai, W.; Ran, Y.; Li, Y.; Wang, B.; Zhou, L. Intestinal microbiome and permeability in patients with autoimmune hepatitis. Best Pract. Res. Clin. Gastroenterol. 2017, 31, 669–673.

95. Lin, R.; Zhou, L.; Zhang, J.; Wang, B. Abnormal intestinal permeability and microbiota in patients with autoimmune hepatitis. Int. J. Clin. Exp. Pathol. 2015, 8, 5153–5160.

96. Wei, Y.; Li, Y.; Yan, L.; Sun, C.; Miao, Q.; Wang, Q.; Xiao, X.; Lian, M.; Li, B.; Chen, Y.; et al. Alterations of gut microbiome in autoimmune hepatitis. Gut 2020, 69, 569–577. https://doi.org/10.1136/gutjnl-2018-317836.

97. Elsherbiny, N.M.; Rammadan, M.; Hassan, E.A.; Ali, M.E.; El-Rehim, A.S.A.; Abbas, W.A.; Abozaid, M.A.A.; Hassanin, E.; Hetta, H.F. Autoimmune Hepatitis: Shifts in Gut Microbiota and Metabolic Pathways among Egyptian Patients. Microorganisms 2020, 8, 1011. https://doi.org/10.3390/microorganisms8071011.

98. Liwinski, T.; Casar, C.; Ruehlmann, M.C.; Bang, C.; Sebode, M.; Hohenester, S.; Denk, G.; Lieb, W.; Lohse, A.W.; Franke, A.; et al. Disease-specific decline of the relative abundance of Bifidobacterium in patients with autoimmune hepatitis. Aliment. Pharmacol. Ther. 2020, 51, 1417–1428. https://doi.org/10.1111/apt.15754.

99. Lou, J.; Jiang, Y.; Rao, B.; Li, A.; Ding, S.; Yan, H.; Zhou, H.; Liu, Z.; Shi, Q.; Cui, G.; et al. Fecal Microbiome Distinguish Patients With Autoimmune Hepatitis From Healthy Individuals. Front. Cell. Infect. Microbiol. 2020, 10, 342. https://doi.org/10.3389/fcimb.2020.00342.

100. Chapman, M.H.; Thorburn, D.; Hirschfield, G.M.; Webster, G.G.J.; Rushbrook, S.M.; Alexander, G.; Collier, J.; Dyson, J.K.; Jones, D.E.J.; Patanwala, I.; et al. British Society of Gastroenterology and UK-PSC guidelines for the diagnosis and management of primary sclerosing cholangitis. Gut 2019, 68, 1356–1378.

101. Saffiotti, F.; Gurusamy, K.S.; Hawkins, N.; Toon, C.D.; Tscharatsiz, E.; Davidson, B.R.; Thorburn, D. Pharmacological interventions for primary sclerosing cholangitis. Cochrane Database Syst. Rev. 2017, 3, CD011343. https://doi.org/10.1002/14651858.cd011343.pub2.

102. Dean, G.; Hanauer, S.; Levitsky, J. The Role of the Intestine in the Pathogenesis of Primary Sclerosing Cholangitis: Evidence and Therapeutic Implications. Hepatology 2020, 72, 1127–1138. https://doi.org/10.1002/hep.31311.

103. Hov, J.R.; Karlsten, T.H. The Microbiome in Primary Sclerosing Cholangitis: Current Evidence and Potential Concepts. Semin. Liver Dis. 2017, 37, 314–331. https://doi.org/10.1053/s-0037-1608801.

104. Nakamoto, N.; Sasaki, N.; Aoki, R.; Miyamoto, K.; Suda, W.; Teratani, T.; Suzuki, T.; Koya, Y.; Chu, P.-S.; Taniki, N.; et al. Gut pathobionts underlie intestinal barrier dysfunction and liver T helper 17 cell immune response in primary sclerosing cholangitis. Nat. Microbiol. 2019, 4, 492–503. https://doi.org/10.1038/s41564-018-0333-1.

105. Tabibian, J.H.; O’Hara, S.P.; Trussoni, C.E.; Tietz, P.S.; Splinter, P.L.; Mounajjed, T.; Hagey, L.R.; LaRusso, N.F. Absence of the intestinal microbiota exacerbates hepatobiliary disease in a murine model of primary sclerosing cholangitis. Hepatology 2016, 63, 185–196. https://doi.org/10.1002/hep.27927.

106. Liao, L.; Schneider, K.M.; Galvez, E.J.C.; Frensen, M.; Marschall, H.-U.; Su, H.; Hatting, M.; Wahlsström, A.; Haybaec, J.; Puchalski, P.; et al. Intestinal dysbiosis augments liver disease progression via NLRP3 in a murine model of primary sclerosing cholangitis. Gut 2019, 68, 1477–1492. https://doi.org/10.1136/gutjnl-2018-316670.

107. Torres, J.; Bao, X.; Goel, A.; Colombel, J.-F.; Pekow, J.; Jabri, B.; Williams, K.M.; Castillo, A.; Odin, J.A.; Meckel, K.; et al. The features of mucosa-associated microbiota in primary sclerosing cholangitis. Aliment. Pharmacol. Ther. 2016, 43, 790–801. https://doi.org/10.1111/apt.13552.

108. Rossen, N.G.; Fuentes, S.; Boonstra, K.; D’Haens, G.R.; Heilig, H.G.; Zoetendal, E.G.; De Vos, W.M.; Ponsioen, C.Y. The Mucosa-associated Microbiota of PSC Patients Is Characterized by Low Diversity and Low Abundance of Uncultured Clostridiales II. J. Crohn’s Colitis 2015, 9, 342–348. https://doi.org/10.1093/cccj/ccu023.

109. Kummer, M.; Thingholm, L.B.; Rühlmann, M.C.; Holm, K.; Hansen, S.H.; Moininho-Silva, L.; Liwinski, T.; Zenouzi, R.; Storm Larsen, C.; Midttun, Ø.; et al. Altered Gut Microbial Metabolism of Essential Nutrients in Primary Sclerosing Cholangitis. Gastroenterology 2020, 160, 1784–1798. https://doi.org/10.1053/j.gastro.2020.12.058.
110. Kummén, M.; Holm, K.; Anmarkrud, J.A.; Nyygard, S.; Vesterhus, M.; Heivik, M.L.; Troseid, M.; Marschall, H.-U.; Schrumpf, E.; Moum, B.; et al. The gut microbial profile in patients with primary sclerosing cholangitis is distinct from patients with ulcerative colitis without biliary disease and healthy controls. *Gut* **2017**, *66*, 611–619. https://doi.org/10.1136/gutjnl-2015-310500.

111. Sabino, J.; Vieira-Silva, S.; Machiels, K.; Joossens, M.; Falony, G.; Ballet, V.; Ferrante, M.; Van Assche, G.; Van der Meersse, S.; Vermeire, S.; et al. Primary sclerosing cholangitis is characterised by intestinal dysbiosis independent from IBD. *Gut* **2016**, *65*, 1681–1689. https://doi.org/10.1136/gutjnl-2015-311004.

112. Kevans, D.; Tyler, A.D.; Holm, K.; Jørgensen, K.K.; Vatn, M.H.; Karlsen, T.H.; Kaplan, G.G.; Eksteen, B.; Gevers, D.; Hov, J.R.; et al. Characterization of Intestinal Microbiota in Ulcerative Colitis Patients With and Without Primary Sclerosing Cholangitis. *J. Crohn’s Colitis* **2016**, *10*, 330–337. https://doi.org/10.1093/ecco-jcc/jjv204.

113. Bajer, L.; Kverka, M.; Kostovic, M.; Macinga, P.; Dvorak, J.; Stehlíkova, Z.; Brezina, J.; Wohl, P.; Spicak, J.; Drastich, P. Distinct gut microbiota profiles in patients with primary sclerosing cholangitis and ulcerative colitis. *World J. Gastroenterol.* **2017**, *23*, 4548–4558. https://doi.org/10.3748/wjg.v23.i25.4548.

114. Lemoine, S.; Kemgang, A.; Ben Belkacem, K.; Straube, M.; Jegou, S.; Corpechot, C.; Chazouillères, O.; Houssset, C.; Sokol, H.; Lemoine, S.; et al. Fungi participate in the dysbiosis of gut microbiota in patients with primary sclerosing cholangitis. *Gut* **2020**, *69*, 92–102. https://doi.org/10.1136/gutjnl-2018-317791.

115. Lapidot, Y.; Amir, A.; Ben-Simon, S.; Veitsman, E.; Cohen-Ezra, O.; Davidov, Y.; Weiss, P.; Bradichevski, T.; Segev, S.; Koren, O.; et al. Alterations of the salivary and fecal microbiome in patients with primary sclerosing cholangitis. *Hepatol. Int.* **2020**, *14*, 191–201. https://doi.org/10.1007/s12072-020-10089-z.

116. Rühlemann, M.; Liwinski, T.; Heinsen, F.-A.; Bang, C.; Zenouzi, R.; Kummén, M.; Thingholm, L.; Tempel, M.; Lieb, W.; Karlsen, T.; et al. Consistent alterations in faecal microorganisms in patients with primary sclerosing cholangitis independent of associated colitis. *Aliment. Pharmacol. Ther.* **2019**, *50*, 580–589. https://doi.org/10.1111/apt.15375.

117. Quraishi, M.N.; Acharjee, A.; Beggs, A.D.; Horniblow, R.; Tselepis, C.; Gkoutos, G.; Ghosh, S.; Rossiter, A.E.; Loman, N.; van Schaik, W.; et al. A Pilot Integrative Analysis of Colonic Gene Expression, Gut Microbiota, and Immune Infiltration in Primary Sclerosing Cholangitis-Inflammatory Bowel Disease: Association of Disease With Bile Acid Pathways. *J. Crohn’s Colitis* **2020**, *14*, 933–947. https://doi.org/10.3748/wjg.v23.i25.4548.

118. Olsson, R.; Björnsson, E.; Bäckman, L.; Friman, S.; Höckersted, K.; Kaijser, B.; Olausson, M. Bile duct bacterial isolates in primary sclerosing cholangitis: A study of explanted livers. *J. Hepatol.* **1998**, *28*, 426–432. https://doi.org/10.1016/S0168-8278(98)03164-4.

119. Björnsson, E.; Kilander, A.; Olsson, R. Bile duct bacterial isolates in primary sclerosing cholangitis and certain other forms of cholestasis—a study of bile cultures from ERCP. *Hepatogastroenterology* **2000**, *47*, 1504–1508.

120. Fosleraa, T.; Melum, E.; Rausch, P.; Juran, B.D.; Ellinghaus, E.; Shiryaev, A.; Laerdahl, J.K.; Ellinghaus, D.; Schramm, C.; Weismüller, T.; et al. Consistent alterations in faecal microorganisms in patients with primary sclerosing cholangitis independent of associated colitis. *Aliment. Pharmacol. Ther.* **2019**, *50*, 580–589. https://doi.org/10.1111/apt.15375.

121. Quraishi, M.N.; Acharjee, A.; Beggs, A.D.; Horniblow, R.; Tselepis, C.; Gkoutos, G.; Ghosh, S.; Rossiter, A.E.; Loman, N.; van Schaik, W.; et al. A Pilot Integrative Analysis of Colonic Gene Expression, Gut Microbiota, and Immune Infiltration in Primary Sclerosing Cholangitis-Inflammatory Bowel Disease: Association of Disease With Bile Acid Pathways. *J. Crohn’s Colitis* **2020**, *14*, 933–947. https://doi.org/10.3748/wjg.v23.i25.4548.

122. Tyc, O.; Jansen, C.; Schierwagen, R.; Uschner, F.E.; Israelens, M.; Klein, S.; Ortiz, C.; Strassburg, C.P.; Zeuzem, S.; Gu, W.; et al. Variation in Bile Microbiome by the Etiology of Cholestatic Liver Disease. *Liver Transplant.* **2020**, *26*, 1652–1657. https://doi.org/10.1002/lt.28882.

123. Tabbibian, J.H.; Weeding, E.; Jørgensen, R.A.; Petz, J.L.; Keach, J.C.; Talwalkar, J.A.; Lindor, K.D. Randomised clinical trial: Vancomycin or metronidazole in patients with primary sclerosing cholangitis—A pilot study. *Aliment. Pharmacol. Ther.* **2013**, *37*, 604–612. https://doi.org/10.1111/apt.12232.

124. Färrkilä, M.; Karvonen, A.-L.; Nurmi, H.; Nuutinen, H.; Taavitsainen, M.; Pikkarainen, P.; Kärkkäinen, P. Metronidazole and ursodeoxycholic acid for primary sclerosing cholangitis: A randomized placebo-controlled trial. *Hepatology* **2004**, *39*, 1379–1386. https://doi.org/10.1002/hep.20457.

125. Davies, Y.K.; Cox, K.M.; Abdullah, B.A.; Safa, A.; Terry, A.B.; Cox, K.L. Long-term Treatment of Primary Sclerosing Cholangitis in Children With Oral Vancomycin: An Immunomodulating Antibiotic. *J. Pediatr. Gastroenterol. Nutr.* **2008**, *47*, 61–67. https://doi.org/10.1097/mpg.0b013e31816fec95.

126. Tabbibian, J.H.; Gossard, A.; El-Youssef, M.; Eaton, J.E.; Petz, J.; Jørgensen, R.; Enders, F.B.; Tabbibian, A.; Lindor, K.D. Prospective Clinical Trial of Rifaximin Therapy for Patients With Primary Sclerosing Cholangitis. *Am. J. Ther.* **2017**, *24*, e56–e63. https://doi.org/10.1097/mjt.0000000000000102.

127. Allegretti, J.R.; Kassam, Z. Fecal Microbiota Transplantation in Patients With Primary Sclerosing Cholangitis: The Next Steps in This Promising Story. *Am. J. Gastroenterol.* **2019**, *114*, 1354–1355. https://doi.org/10.1038/s41393-019-02931-w.

128. Bajaj, J.S.; Fagan, A.; Sikaroodi, M.; White, M.B.; Sterling, R.K.; Gilles, H.C.; Heuman, D.; Stravitz, R.T.; Matherly, S.C.; Siddiqui, M.S.; et al. Liver transplant modulates gut microbial dysbiosis and cognitive function in cirrhosis. *Liver Transplant.* **2017**, *23*, 907–914. https://doi.org/10.1002/lt.24754.

129. Bajaj, J.S.; Kakiyama, G.; Cox, J.I.; Nittono, H.; Takei, H.; White, M.; Fagan, A.; Gavis, E.A.; Heuman, D.M.; Gilles, H.C.; et al. Alterations in gut microbial function following liver transplant. *Liver Transplant.* **2018**, *24*, 752–761. https://doi.org/10.1002/lt.25046.
130. Sun, L.Y.; Yang, Y.-S.; Qu, W.; Zhu, Z.-J.; Wei, L.; Ye, Z.-S.; Zhang, J.-R.; Sun, X.-Y.; Zeng, Z.-G. Gut microbiota of liver transplantation recipients. Sci. Rep. 2017, 7, 376. https://doi.org/10.1038/s41598-017-03476-4.

131. Annavajhala, M.K.; Gomez-Simmonds, A.; Macesic, N.; Sullivan, S.B.; Kress, A.; Khan, S.D.; Giddins, M.I.; Stumpf, S.; Kim, G.I.; Narain, R.; et al. Colonizing multidrug-resistant bacteria and the longitudinal evolution of the intestinal microbiome after liver transplantation. Nat. Commun. 2019, 10, 1–12. https://doi.org/10.1038/s41467-019-12633-4.

132. Kriss, M.; Verna, E.C.; Rosen, H.R.; Lozupone, C.A. Functional Microbiomics in Liver Transplantation: Identifying Novel Targets for Improving Allograft Outcomes. Transplantation 2019, 103, 668–678. https://doi.org/10.1097/tp.0000000000002568.

133. Corbitt, N.; Kimura, S.; Isse, K.; Specht, S.; Chedwick, L.; Rosborough, B.R.; Lunz, J.G.; Murase, N.; Yokota, S.; Demetris, A.J. Gut Bacteria Drive Kupffer Cell Expansion via MAMP-Mediated ICAM-1 Induction on Sinusoidal Endothelium and Influence Preservation-Reperfusion Injury after Orthotopic Liver Transplantation. Am. J. Pathol. 2013, 182, 180–191. https://doi.org/10.1016/j.ajpath.2012.09.010.

134. Xing, H.C.; Li, J.L.; Xu, K.J.; Shen, T.; Chen, Y.B.; Sheng, J.F.; Chen, Y.; Fu, S.Z.; Chen, C.L.; Wang, J.G.; et al. Protective role of supplement with foreign Bifidobacterium and Lactobacillus in experimental hepatic ischemia-reperfusion injury. J. Gastroenterol. Hepatol. 2006, 21, 647–656. https://doi.org/10.1111/j.1440-1446.2006.04306.x.

135. Ren, Z.; Cui, G.; Lu, H.; Chen, X.; Jiang, J.; Liu, H.; He, Y.; Ding, S.; Hu, Z.; Wang, W.; et al. Liver Ischemic Preconditioning (IPC) Improves Intestinal Microbiota Following Liver Transplantation in Rats through 16s rDNA-Based Analysis of Microbial Structure Shift. PLoS ONE 2013, 8, e75950. https://doi.org/10.1371/journal.pone.0075950.

136. Ito, T.; Nakamura, K.; Kageyama, S.; Korayem, I.M.; Hirao, H.; Kadono, K.; Aziz, J.; Younan, S.; DiNorića, J.; Agopian, V.G.; et al. Impact of Rifaximin Therapy on Ischemia/Reperfusion Injury in Liver Transplantation: A Propensity Score–Matched Analysis. Liver Transplant. 2019, 25, 1778–1789. https://doi.org/10.1002/lt.25633.

137. Grańt, M.; Wronka, K.M.; Lewandowski, Z.; Grańt, K.; Krasnodębski, M.; Stypułkowski, J.; Hokówko, W.; Masior, Ł.; Kosińska, S.; Wasilewicz, M.; et al. Effects of continuous use of probiotics before liver transplantation: A randomized, double-blind, placebo-controlled trial. Clin. Nutr. 2017, 36, 1530–1539. https://doi.org/10.1016/j.clnu.2017.04.021.

138. Ren, Z.; Jiang, J.; Lu, H.; Chen, X.; He, Y.; Zhang, H.; Xie, H.; Wang, W.; Zheng, S.; Zhou, L. Intestinal Microbial Variation May Predict Early Acute Rejection after Liver Transplantation in Rats. Transplantation 2014, 98, 844–852. https://doi.org/10.1097/tp.0000000000003343.

139. Xie, Y.; Luo, Z.; Li, Z.; Deng, M.; Liu, H.; Zhu, B.; Ruan, B.; Li, L. Structural Shifts of Fecal Microbial Communities in Rats with Acute Rejection after Liver Transplantation. Microb. Ecol. 2012, 64, 546–554. https://doi.org/10.1007/s00248-012-0030-1.

140. Kato, K.; Nagao, M.; Miyamoto, K.; Oka, K.; Takahashi, M.; Yamamoto, M.; Matsumura, Y.; Kaido, T.; Uemoto, S.; Ichiyama, S. Longitudinal Analysis of the Intestinal Microbiota in Liver Transplantation. Transplant. Direct 2017, 3, e144. https://doi.org/10.1097/txd.0000000000000661.

141. Sawas, T.; Al Halabi, S.; Hernaez, R.; Carey, W.D.; Cho, W.K. Patients Receiving Prebiotics and Probiotics Before Liver Transplantation Develop Fewer Infections Than Controls: A Systematic Review and Meta-Analysis. Clin. Gastroenterol. Hepatol. 2015, 13, 1567–1573.e3. https://doi.org/10.1016/j.cgh.2015.05.027.

142. Zhang, J.; Ren, F.G.; Liu, P.; Zhang, H.K.; Zhu, H.Y.; Feng, Z.; Zhang, X.F.; Wang, B.; Liu, X.M.; Zhang, X.G.; et al. Characteristics of fecal microbial communities in patients with non-anastomotic biliary strictures after liver transplantation. World J. Gastroenterol. 2017, 23, 8217–8226. https://doi.org/10.3748/wjg.v23.i46.8217.

143. Satapathy, S.K.; Banerjee, P.; Pierre, J.F.; Higgins, D.; Dutta, S.; Heda, R.; Khan, S.D.; Mupparaju, V.K.; Mas, V.; Nair, S.; et al. Characterization of Gut Microbiome in Liver Transplant Recipients With Nonalcoholic Steatohepatitis. Transplant. Direct 2020, 6, e625. https://doi.org/10.1097/txd.0000000000001033.

144. Kipp, F.; Kabar, I.; Hüsing, A.; Cicinnati, V.R.; Heitschmidt, L.; Beckebaum, S.; Thölking, G.; Schmidt, H.H.; Karch, H. Analysis of Bile Colonization and Intestinal Flora may Improve Management in Liver Transplant Recipients Undergoing ERCP. Ann. Transplant. 2015, 20, 249–255. https://doi.org/10.1097/txd.0000000000000333.

145. Liu, Y.; Sun, L.-Y.; Zhu, Z.-J.; Wei, L.; Qu, W.; Zeng, Z.-G. Bile microbiota: New insights into biliary complications in liver transplant recipients. Ann. Transl. Med. 2020, 8, 354–354. https://doi.org/10.21037/atm.2020.02.60.

146. Liu, Y.; Jia, J.-D.; Sun, L.-Y.; Zhu, Z.-J.; Zhang, J.-R.; Wei, L.; Qu, W.; Zeng, Z.-G. Characteristics of bile microbiota in liver transplant recipients with biliary injury—PubMed. Int. J. Clin. Exp. Pathol. 2018, 11, 481–489.