Review

Systematic Review of the Status of Microbiota in Cirrhosis. A Change Towards a More Pathogenic Predisposition

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Review

A Systematic Review for Microbiota in Cirrhosis: A Change Towards a More Pathogenic Predisposition

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Abstract: Background: The microbiome of the human intestine is a regulator of health by modulating immune response and playing important role in metabolism. Dysbiosis, diversity and abundance of microbiota communities in the gut has shown changes in cirrhosis and its complications. **Aim:** To review current knowledge regarding microbiota alterations in cirrhosis, its potential differences according to etiology and its role in the development of cirrhosis complications. **Methods:** A systematic search of online bibliographic database up to July 2024 was performed. Randomized controlled trials, observational and cohort studies that included in total or at least a cohort of cirrhotic adult patients were enlisted for data extraction and analysis. **Results:** A total of 73 publications were included for data extraction. Alpha diversity was found decreased in cirrhotic patients in 30/38 (78%) of the studies, while beta diversity 20/22 (90%) presented significant difference between healthy and cirrhotic groups. Proteobacteria were significantly increased in 20/27 (74%) studies followed by Actinobacteria and Fusobacteria while 22/25 (88%) studies found either reduction in cirrhotic patients or increased abundance in healthy controls for Firmicutes and Bacteroidetes. The most abundant genera in hepatic encephalopathy groups were pathobionts such as Enterococcus and Streptococcus followed by Vellionella and Escherichia. Heterogeneity was found among studies regarding Alpha-diversity in Hepatocellular Carcinoma (HCC) as it was decreased in 3 studies, indifferent in 5 and increased in 3 in comparison to cirrhotic non-HCC patients. **Conclusion:** Dysbiosis of the gut microbiota is associated with cirrhosis and development of complications such as hepatic encephalopathy and hepatocellular carcinoma.

Keywords: cirrhosis; dysbiosis; microbiota; hepatic encephalopathy; hepatocellular carcinoma; bacterial overgrowth

1. Introduction

The liver as an organ has multiple metabolic functions but by staying in between the gut and the rest of human organism exerts also important immune mediated – defensive activities. It has been described to be part of the gut – liver axis due to significant first pass effect over microbes, medical molecules, toxic substances such as alcohol and food(1, 2). Many chronic liver diseases have been developing once this important protective mechanism has been damaged or saturated(1, 3). Additionally, several complications that appear in liver cirrhosis depend on these altered pathophysiological mechanisms(1).

The gut microbiome which grows and resides in all parts of the gut, especially in the small intestine exerts many effects that help or sometimes damage liver function, such as, the metabolism of bile acids(4), alcohol(5), and immune tolerance(3, 6). Even though liver has a high capacity of immune protection and metabolic reactions, these are not unlimited and therefore an intact barrier

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that can control input is necessary(7). This barrier is represented by the gut, which forms a complicate system built on different layers(1). The main layers of this barrier are: the mucus layer, the enteric cellular layer including enterocytes, goblet cells, tuft cells and enterochromaffin cells, the rich immune system layer in between and under the enteric cells and finally the vascular system layer which delivers all contents to the portal vein. In liver diseases significant changes have been described in the production of mucins and short chain fatty acids both controlled by the presence of "healthy" commensal bacteria(1, 6). In addition, a reduction in the population of bacteria containing 7a glucuronidase change the composition of secondary bile acids and induces reactions through FXR receptor(4). Finally, a leaky gut status is caused by microbiota changes which produce an immune mediated reaction that increase turnover of enterocytes and alters the formation of proteins of the tight junctions (5, 8). These are probably the most important pathophysiologic mechanisms that have been described to date.

Dysbiosis, is called every stable change or imbalance in number, diversity and abundance of microbial populations in the gut as from the previous healthy state(9). Dysbiosis is found in early states of many chronic liver diseases before and after the development of cirrhosis(10, 11). All microbiota changes represent an imbalance between protective bacteria like the ones included in the firmicutes taxa and potentially pathogenic bacteria like Proteobacteria (E.Coli, Klebsiella)(10). In addition, in several instants during chronic liver diseases certain microbial populations reach a number that we conventionally describe as bacterial overgrowth(10-14). In cirrhosis a significant factor for the development of bacterial overgrowth is small intestinal dysmotility which seems to be reduced by portal hypertension(15, 16).

Therefore, in the cirrhosis stage especially when portal hypertension has been developed the presence of dysbiosis with or without bacterial overgrowth and leaky gut can cause bacterial translocation and consequently septic episodes of bacterial peritonitis, variceal bleeding and hepatic encephalopathy(1, 8, 10, 12, 16). The purpose of this review is to collect current knowledge regarding microbiota changes in cirrhosis, its differences in comparison to etiology and its role on the development of cirrhosis complications.

2. Methods

2.1. Data Identification

We searched PUBMED database from 1970 to 14 of July 2024 for English and non-English publications using text words Gut Microbiota/Gut Flora/Gut Microbioma AND Cirrhosis. We manually searched references from review articles and original studies.

2.2. Inclusion and Exclusion Criteria

Studies included for analysis needed to fulfil the following criteria: (i) published as full paper; (ii) retrospective, prospective or randomized controlled design; (iii) including in total or at least a cohort of cirrhotic patients (iv) no animal studies, (v) involving population of age more or equal to 18 years;

Studies were excluded if: (i) were analyzing or comparing results of a specific treatment on microbiota (ii) groups referring on transplanted patients (iii) had no taxonomic analysis (iv) reporting microbiota other than intestinal (v) focused on fungal or viral species.

2.3. Data Extraction and Methodological Assessment

Data were extracted independently tabulated using a predefined review form. This work was conducted with the PRISMA statement of Preferred Reporting Items for Systematic Reviews and Meta-analyses(17). The flowchart is licensed under CC BY 4.0. Thereafter two authors were assigned for quality assessment. The AMSTAR 2 tool was used to assess the quality of the studies. AMSTAR 2 doesn't involve a scoring system but rather a rating of the overall confidence of the results of the review ranging from high to critically low(18).

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The decision to include or exclude a study was made before the review analysis was carried out and agreed by consensus amongst the authors. In the present review agreement between reviewers for selection of articles for all analyses was 100%.

2.4. End Points

Our endpoints for the present review were to assess changes in microbiota in patients with cirrhosis and additionally make subgroups: 1) according to cirrhosis aetiology (ALD, MASLD, HBV etc.) 2) according to cirrhosis stage or complications (Compensated vs Decompensated, Hepatocellular Carcinoma (HCC), Hepatic Encephalopathy (HE), Spontaneous Bacterial Peritonitis (SBP). Infections, Acute on Chronic Liver Failure (ACLF) 3) According to the sample used (fecal samples, duodenal fluid, rectal swabs or colon biopsies), 4) According to the test used (16S PCR, deep sequencing metagenomics, other) 5) According to geographical distribution. In addition, we checked for papers that report microbiota in cirrhotics vs non cirrhotics of the same aetiology.

3. Results and Discussion

The search strategy found 2207 references that were imported for screening **(Figure 1)**.

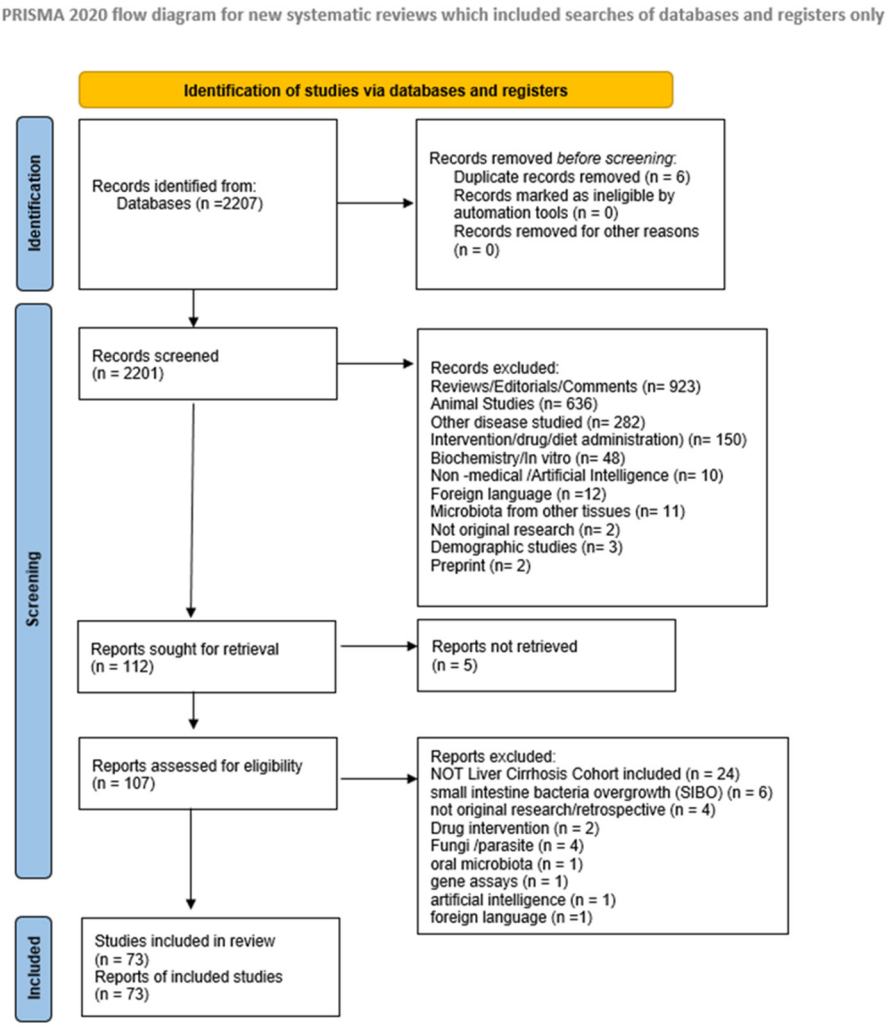

Figure 1. PRISMA Flow Chart excluded and included studies for microbiota changes in cirrhosis.

Six duplicates were found and removed. Studies screened by title and abstract were 2201 and 2095 of them were excluded according to the aforementioned criteria. Of the remaining 122 articles, 5 were excluded due to no full text retrieval. Of the 117 full text articles screened for eligibility 44

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were excluded as 24 did not include a liver cirrhosis group, 4 were not original research, 10 did not report results on microbiota taxonomic differences (4 were about fungi or parasites and 6 reported only the presence of small intestinal bacterial overgrowth) and 6 on not meeting the inclusion criteria (2 included drug intervention, 1 was about oral microbiota, 1 was on artificial intelligence practice, 1 was conducted as a gene assay and 1 was in non-English language).

Finally, we identified 73 studies that met inclusion criteria(12, 15, 19-88). **(Figure 1)** In these 73 studies identified there was at least one cohort of patients diagnosed with cirrhosis. Diagnosis of cirrhosis was confirmed either histologically by liver biopsy or combinations of clinical, laboratory parameters and/or imaging features (abdominal ultrasound/elastography).

In order to compare findings, we also separately analyzed patients with decompensated cirrhosis and complications. Regarding decompensation diagnosis was confirmed by a history of clinical, endoscopy and imaging features of ascites, HE, varices and jaundice. Studies that solely included patients with cirrhosis and HE or HCC were analyzed independently and were not included in the analysis of studies that compared compensated and decompensated patients. Studies that included arms of patients receiving antibiotics or immunomodulating medications were excluded.

3.1. Microbiota Diversity in Cirrhosis

To assess microbiota changes we analyzed the data of 73 studies. A total number of 9.763 individuals was extracted. Of them 2494 were healthy control groups, 5473 were patients with liver cirrhosis, 2494 were patients with chronic liver disease without cirrhosis while 550 were patients with HCC. In regard to geographical distribution 33 studies were from East Asia (China 29, Japan 3, Southern Korea 1) and 3 from South Asia (Taiwan 2, India 1), 21 from North or Central America (USA 19, Canada 1, Mexico 1), 6 from North or Central Europe (Poland 1, UK 1, Germany 1, France 1, Finland 1, Austria 1), and 9 from South or East Europe (Russia 4, Italy 2, Spain 1, Israel 1, Greece 1). **(Figure 2)**

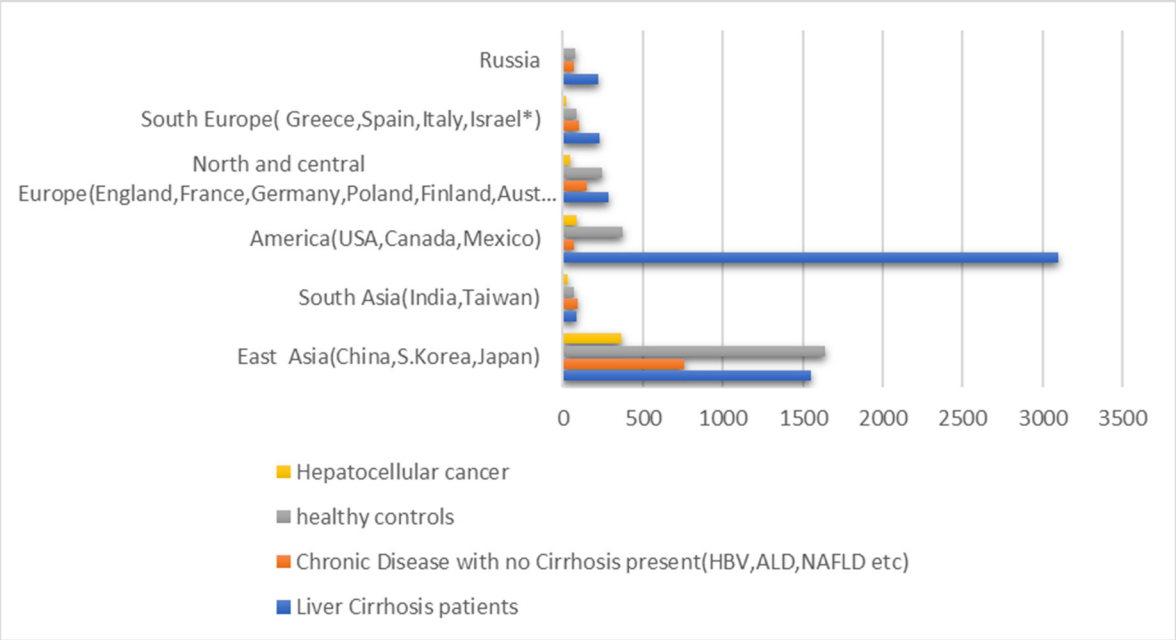

Figure 2. Patients included in the study and their relative groups of etiology according to geographical distribution.

Most studies used fecal samples to identify and quantify human gut microbiota (67/73 91.7%). For the rest 2 studies used duodenal biopsies, another 2 a combination of fecal samples and colon biopsies and 1 rectal swab. In addition, most studies (50/73, 68.4%) used as method of detection the 16s r-RNA sequencing, while much less (8/73, 10.9%) appointed the Shotgun Metagenomics method, and (6/73, 8.2%) the 16s r-DNA sequencing. The less used was PCR and bacterial cultures methods. Almost all studies investigated the entire human gut flora while only one that focused on specific

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species and their role in cirrhosis pathogenesis was included. This Chinese cohort analyzed subspecies of Desulfovibrio and results showed a specific species fairly increase in cirrhotic patients with a significant difference in its trait like desulfurization ability(28).

The diversity and composition data of the gut microbiota available were presented in different manners. In 13 studies results focused on most enriched species detected whereas 53 focused on the most abundant genera, families or phyla and 7 reporting the most enriched bacterial groups. In total 12 phyla, 47 families and 118 genera were reported.

Alpha diversity analysis on most studies was conducted using metrics such as Chao1, Ace, Shannon, and Simpson indices. Of the total 73 studies included 53 (72%) reported results regarding on alpha or beta diversity **(Figure 3)**. Alpha diversity on healthy versus cirrhotic patients was presented in 38 studies. With the term cirrhosis without specific etiology there were 14/38 (37%) studies while as liver cirrhosis with a specific etiology (viral HBV or HVC, or metabolic NASH, or ALD) 24(63%). In 30 studies a significant decrease in alpha diversity was observed (78%) in cirrhosis compared to healthy subjects, 5 showed no difference, and 3 noted an increase in liver cirrhosis groups **(Figure 3)**. However, data from the latter 3 studies collected came in the first from rectal swab(45) indicating a possible different composition in rectal gut flora in comparison to the rest of the colon, the second from a very small Chinese cohort possibly introducing bias(66) and the third was a study from Taiwan compering HBV related cirrhotic patients with their non-cirrhotic counterparts(25). In conclusion despite a few discrepancies, most studies agree that a significant decrease in alpha diversity of the gut microbiota is associated with liver cirrhosis.

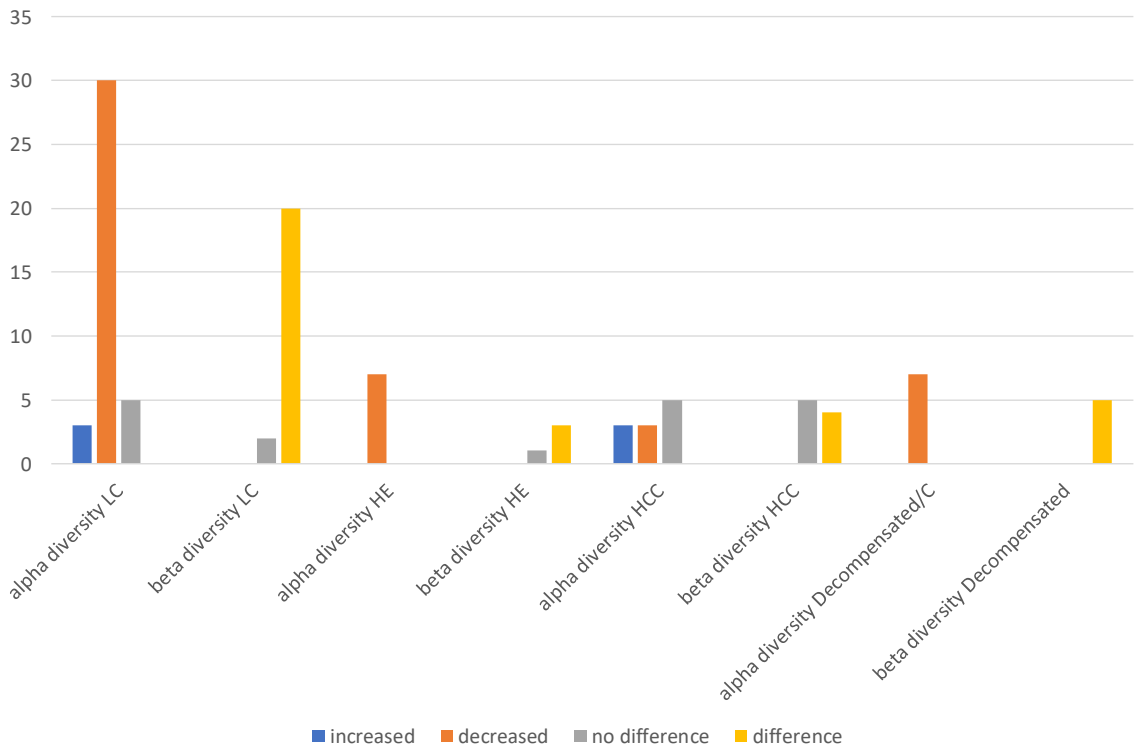

Figure 3. Results from studies reporting alpha and beta diversity of gut microbiota differences of Liver Cirrhosis (LC) patients vs Healthy controls (HC), Cirrhotic patients with vs without hepatic encephalopathy (HE), Cirrhotic patients with vs without hepatocellular cancer (HCC), Cirrhotic patients with vs without decompensation.

In regard to beta diversity, most common metrics used were Bray -Curtis Dissimilarity, weighted and unweighted UniFrac. With the term cirrhosis without specific etiology there were 22 studies presenting results **(Figure 3)**. Of them in 20/22 (90%) a significant difference between healthy and cirrhotic groups was showed while in 3 results were opposing. This particular finding adds to

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the hypothesis that in cirrhosis the intestinal microbiota undergoes significant changes in its composition that needs to be revealed.

3.2. Gut Microbiota Composition in Liver Cirrhosis

The human gut microbiota in cirrhosis is compromised by a plethora of species. Twenty seven studies reported results on phyla level, 21 on family and 44 on genera level in patients with cirrhosis while 25, 22 and 38 respectively reported in patients with no cirrhosis. At the phyla level, proteobacteria seem to play the most important role in gut dysbiosis in cirrhosis, as they were significantly increased in 20/27 (74%) studies followed by Actinobacteria and Fusobacteria **(Figure 4)**.

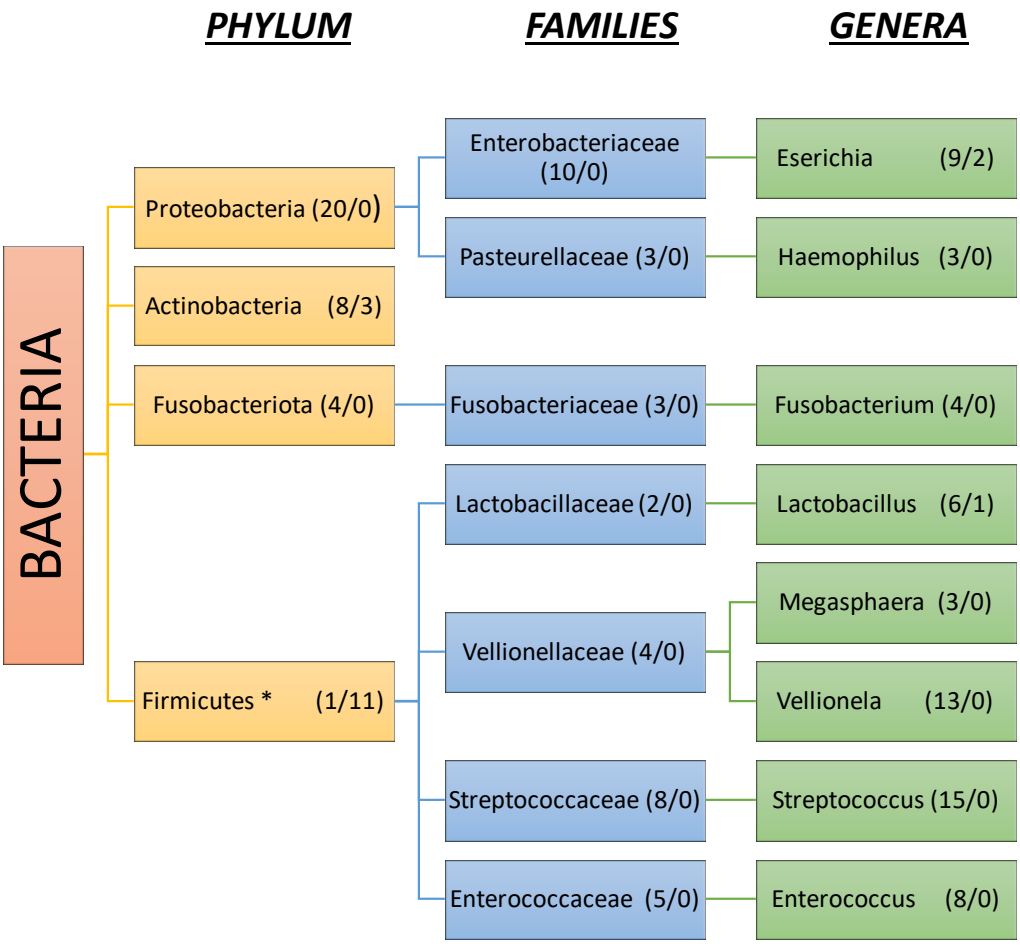

Figure 4. Gut microbiota enriched in Liver Cirrhosis. (number of studies that support enrichment/number of studies that support reduction) * Although abundance of firmicutes is reduced in patients with liver Cirrhosis compared to healthy subjects some families and genera included in this phylum tend to be significantly enriched.

In contrast 22/25 (88%) studies found either reduction in cirrhotic patients or increased abundance in healthy controls for Firmicutes and Bacteroidetes **(Figure 5).** Notably although most results agree on Proteobacteria and Firmicutes changes there seem to be a discrepancy in Bacteroidetes as 11/25 (44%) studies report an increase in healthy-control versus cirrhotic groups while 5/27 (18%) report the opposite. Another important aspect regarding Firmicutes is that at the level of sub-taxa, genera as Streptococcus, Vellionella, and Enterococcus are considerably increased in numbers in cirrhotic patients than in non-cirrhotic subjects.

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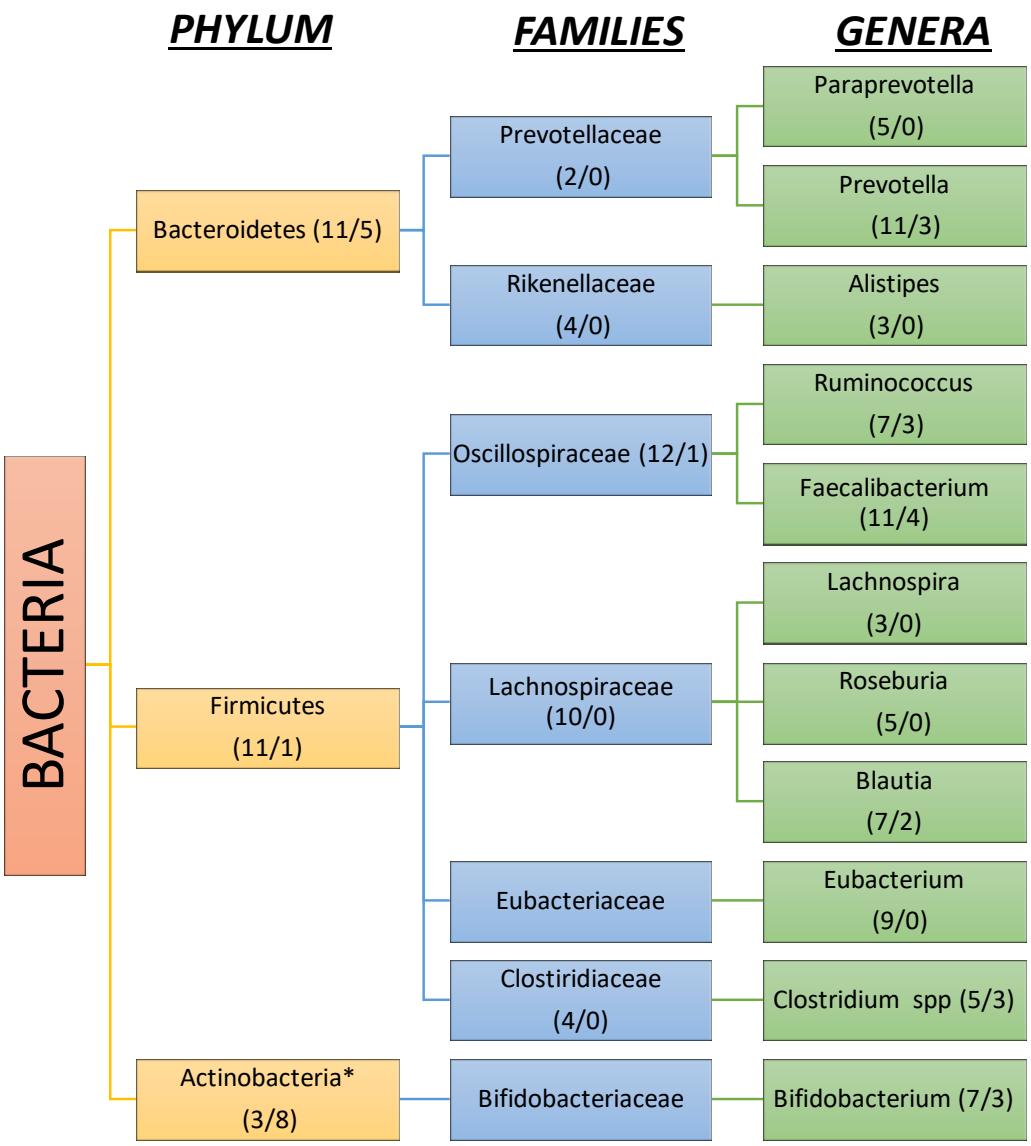

Figure 5. Gut microbiota decreased in Liver Cirrhosis vs Healthy subjects. (number of studies that support reduction/number of studies that support enrichment) * Although abundance of actinobacteria is increased in patients with liver Cirrhosis compared to healthy subjects Bifidobacterium tends to be more enriched in healthy vs cirrhotic patients.

At the family level changes found in liver cirrhosis consist of both an increase in abundance of Enterobacteriaceae, Pasteurellaceae, Fusobacteraceae, Lactobacillaceae, Vellionellaceae, Enterococcaceae and Streptococcaceae, and decrease of Oscillospiraceae, Lachnospiraceae, Clostiridiaceae, Eubacteriaceae, Rikenellaceae and Bifidobacteriaceae. In particular Streptococcaceae and Enterobacteriaceae are the most commonly mentioned as enriched while Oscillospiraceae and Lachnospiraceae the most decreased respectively.

As far as genera are considered, we have found decreases in autochthonous taxa such as Prevotella, Paraprevotella, Roseburia, Faecalibacterium, Eubacterium, and Ruminococcus. Otherwise, conflicting results are been presented among studies regarding Bifidobacterium, Clostiridium spp, and Bacteroides with most of them been in favor of decrease and only in certain cases an increased abundance is presented. A possible explanation for this heterogeneity regards the fact that different etiologies of chronic liver diseases such as ALD, NAFLD, HBV may influence microbiota even before progression into cirrhosis and remain as such thereafter. On the other hand, genera that are largely facultative anaerobes such as Enterococcus, Escherichia, Klebsiella, Lactobacillus, Streptococcus and Haemophilus are been increasing in cirrhosis. Vellionella an oral

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cavity bacterial species is also significantly increased in cirrhotic patients. Finally, another important finding is that Rumminococcus Gnavus group has been linked to cirrhosis in at least 3 studies when most Rumminococcus spp are significantly decreasing in the cirrhotic gut environment (23, 26, 85).

3.3. Gut Microbiota Composition in Hepatic Encephalopathy

Hepatic encephalopathy (HE), characterized by a broad spectrum of neuropsychiatric symptoms, is a major complication in decompensated cirrhosis(89). It can be classified as Grade I, covert and subsequently minimal HE (recognized by specific cognitive tests), and overt ranging from grade II to IV exhibiting severe neuropsychiatric manifestations(90). Most studies focused on minimal HE using different scores for diagnosis such as PHES, NCT-A, DST. We found 16 studies in total which appointed results for HE either regarding taxonomic (5 on phyla, 7 on families and 11 on genera) or diversity (7 on alpha 4 on beta diversity). Alpha diversity in cirrhotic patients with HE was reported in 7/7(100%) of the studies as decreased in comparison to cirrhotic patients without HE.

The main phyla that were enriched in HE patients were Proteobacteria, whereas for families there were Streptococcaceae, Lactobacillaceae, Enterobacteriaceae, Enterococcaceae, and Staphylococceae. The most abundant genera were pathobionts such as Enterococcus and Streptococcus followed by Vellionella and Escherichia(20, 26, 41, 43, 51, 54, 65, 75, 77, 86) **(Figure 6)**. Notably, in a Chinese study Streptococcus Salivarius was strongly associated with the presence of HE in cirrhotic patients(77). Another interesting finding was that the presence of Bifidobacterium an autochthonous taxon with beneficial effects was reported to be enriched in 3 studies (51, 75, 82). A study from USA associated Alcaligeneceae, Porphyromonadaceae with worse cognition(65). In order to evaluate microbiota composition in MHE a study demonstrated results from cirrhotic patients that were subjected to three types of cognitive tests and the results although not completely matching had similarities regarding some bacterial groups that were enriched such as bacteria from the Lactobacillaceae family(72).

	PHYLUM	***FAMLILES***	***GENERA***
HCC patients increased abundance vs non HCC	Proteobacteria (5/0) Fusobacteria (3/0) Actinobacteria (3/1)		Streptococcus (3/0) Paraprevotella (3/0) Haemophilus (2/0) Enterococcus (2/0) Akkermansia (2/0) Vellionela (2/1)
Non-HCC patients increased abundance vs HCC	Verrucomicrobia (2/0)	Lachnospiraceae (2/0) Ruminococcaceae (2/1)	Ruminococcus (3/1) Blautia (2/0)
HE patients increased abundance vs non HE	Proteobacteria (4/0)	Streptococcaceae (3/0) Lactobacillaceae (4/0) Enterobacteriaceae (3/0) Enterococcaceae (2/0) Staphylococcae (2/0)	Bifidobacterium (3/0) Enterococcus (5/0) Escherichia (3/0) Streptococcus (4/0) Vellionela (3/1)
Non-HE cirrhotic patients increased abundance vs HE		Lachnospiraceae (7/0) Clostridiaceae (6/0) Ruminococcaceae (4/0)	Bacteroides (3/1) Roseburia (2/0) Ruminococcus (3/0) Lachnospira (2/0) Eubacterium (2/0) Anaerostipes (2/0)

Figure 6. Comparison of hepatic encephalopathy and Hepatocellular Carcinoma most presented bacteria taxa in the studies. (number of studies that show increase/number of studies that show no increase or decrease). Taxa that are reported in the results of at least two or more studies are presented in the graph.

On the contrary some bacteria that were decreased in patients with HE compared to cirrhotic patients without encephalopathy are members from the families Lachnospiraceae, Clostridiaceae,

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Ruminococcaceae such as Bacteroides, Roseburia, Ruminococcus, Lachnospira and Eubacterium(20, 26, 41, 43, 51, 60, 65, 72, 75, 77, 80, 81) **(Figure 6).**

3.4. Gut Microbiota Composition in Hepatocellular Carcinoma

Epidemiological studies have shown that 70% to 90% of hepatocellular carcinoma cases are found in patients with preceding cirrhosis or advanced fibrosis(91). We identified 13 studies reporting microbiota alterations in patients with HCC **(Figure 6)**. Alpha diversity showed a notable heterogeneity and it was found to be decreased in 3 studies(27, 58, 92) but increased in 3(40, 59, 74) while 5 of them(30, 44, 55, 71, 83) showed no differences in comparison to cirrhotic patients without HCC. These results may derive from the difference in populations of microbiota gut between patients that develop HCC in the presence of cirrhosis in oppose to those that don't as some studies show(40, 58). Additionally, beta diversity showed no difference between groups in 6 studies(30, 40, 44, 55, 59, 71) whereas 4 studies showed dissimilarity(27, 46, 58, 92).

Considering gut flora composition, at phyla level changes include increase in proteobacteria, fusobacteria and actinobacteria and at family level some beneficial taxa like Lachnospiraceae and Rumminococcaceae belonging to the Firmicutes decreased. Genera that proved enriched were Paraprevotella, Akkermansia, Haemophilus and pathobionts such as Streptococcus and Enterococcus while Ruminococcus, Blautia and Bifidobacterium were decreased. Taken together the above findings suggest a significant increase in pathogens like proteobacteria and Streptococcaceae and on the other hand a decrease in beneficial bacteria such as those that reduce hydrophobic bile acids. As the role of pathogens in the development of HCC is not clear a Polish study in patients with liver cirrhosis undergoing liver transplant found a significant correlation between fecal E. Coli and the presence of HCC(68). In addition, while Lactobacillus is known to be beneficial in human homeostasis, as it belongs to a group of certain bacterial genera that are involved in bile acid deconjugation, oxidation/epimerization, and 7-dehydroxylation together with Bacteroides, Bifidobacterium, Ruminococcus, and Clostridium(93) a study from Germany on NASH associated the presence of HCC in cirrhotic patients with increased abundance in the Lactobacillus species (58). **(Figure 6).**

3.5. Gut Microbiota Composition in Other Complications

Few studies assessed microbiota changes and other complications such as ascites, spontaneous bacterial peritonitis (SBP), infections or ACLF(31, 32, 34, 38, 47, 48, 53, 57, 82, 86). Regarding microbiota changes during the passage from compensated to decompensated cirrhosis(31, 36, 40, 42, 55-57), or based on Child-Pugh score(64, 70), alpha diversity was found to be decreased in all studies **(Figure 3).** Most abundant bacterial groups were at Enterococcaceae, Streptococcace and Peptostreptococcaceae families while Enterococcus was the most prominent genera followed by Streptococcus, Staphylococcus, Vellionella and Lactobacillus. These findings follow the pattern already present with pathobionts increasing as the liver disease progresses.

Clinically significant ascites is associated with increase in Proteobacteria and decrease in Actinobacteria and Bacteroidetes as one particular study from Russia demonstrated(32). Very few studies focused on SBP including one from Russia that showed enrichment in Proteobacteria, and particularly Gammaproteobacteria including pathogens like Klebsiella, Serratia, Acinetobacter and Moraxella spp (31) and a study from India in cirrhotic patients with infections that found in SBP enrichment of Oxalobacteraceae, Neisseriaceae, Anaerotruncus(34). Gammaproteobacteria, Lactobacillaceae and Enterobacteriaceae were found enriched in patients with concomitant infections such as pneumonia, bacteremia, cholangitis but results were sparse among different infections(34, 38, 65, 86).

Finally, in ACLF enterococcus spp was found to be the most prominent taxa(48, 57), while in another study from USA Proteobacteria, Actionobacteria, Cambylobacteracae were most abundant(82).

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3.6. Gut Microbiota Assessment in Liver Cirrhosis Various Etiologies

Of the studies reviewed 11 were conducted on HBV, 7 on ALD and 6 on NAFLD populations specifically. As already mentioned, alpha diversity is mostly decreased in cirrhotic patients and this pattern had no exception between different etiologies. Specifically of the 8 studies that were conducted on HBV cirrhotic patients reporting results on alpha -diversity 6 indicated decrease(27, 36, 42, 48, 52, 56, 92), 1 indifferent(29) and 1 increase(25) while 5 reported differences in beta diversity versus non-cirrhotic controls. Regarding NAFLD 5 reported decreased alpha diversity and high beta diversity index(58, 69, 71, 85, 88). In ALD 4 studies presented decreased alpha diversity(40, 49, 59, 61) while only 2 showed no significant difference(73, 87).

The main phyla enriched were Actinobacteria and Proteobacteria in HBV cirrhotic populations presented in 4 and 5 studies respectively, Actinobacteria, Proteobacteria and Fusobacteria in ALD and Proteobacteria mainly in NAFLD cirrhotic populations. Most prevalent families were Enterobacteriaceae in all aetiologies (27, 39, 42, 49, 56, 63, 71, 88) and Streptococcaceae in HBV populations(19, 27, 42, 56). Notably Streptococcus and Vellionella species were presented in 6(19, 27, 28, 36, 56, 92) and 5 studies(25, 27, 56, 62, 92) respectively and were the most increased genera in HBV cirrhotic populations while Lactobaccilus was significantly increased in ALD groups(44, 59, 87).

In the present systematic review we collected all published studies that identify the status of microbiota in cirrhosis. Our findings show that cirrhotic patients have significant changes in both alpha and beta diversity compared to controls which in most studies are healthy volunteers. Changes refer to all taxa of bacteria namely phylum, families and genera. At the level of phylum, we have conflicting results showing both increase and decrease in abundance but at a lower level we have found specific changes in families and genera. We conclude that at Proteobacteria phulum, Enterobacteriaceae and Pasteurellaceae families increase, at Firmicutes phylum, Lactobacillaceae Velionellaceae Streptococcacceae and Enterococcaceae increase whilst Oscillospiraceae, Lachnospiraceae, Eubacteriaceae and Clostiridiaceae decrease. At Bacteriodetes phylum, Prevotelacceae and Rikenellaceae decrease, at Actinobacteria, Bifidobacteriaceae decrease. Finally at Fusobacteria phylum, Fusobacteriaceae are increasing. Another important finding of this review is that as cirrhosis progress to a decompensated stage and complications appear there is a prominent change in alpha and beta diversity as well. However, until now the complication mostly studied is hepatic encephalopathy. In hepatocellular carcinoma studies show conflicting results regarding alpha and beta diversity.

A normal gut microbiota consists of 6 phyla namely Proteobacteria, Firmicutes, Bacteroidetes, Actinobacteria, Fusobacteria and Verrucomicrobia with the phyla Bacteroidetes and Firmicutes representing more or less 90% of the population(94). Normal distribution of gut microbiota in the intestinal lumen is associated with different pH values, motility, oxygen levels and other conditions in the environment. Stomach is dominated by oral cavity bacteria such as Vellionella, Streptococcus, Prevotella, Rothia, Haemophilus (95), dudodenum mostly facultative anaerobic due to its hostile environment and jejunum gram positive aerobic as well as facultative anaerobic like Lactobacillus, Enterococcus and Streptococcus(96). In the ileum, the main bulk consist of Enterococcus, Bacteroides, Lactobacillus, Clostridium, Corynebacteria while the cecum and the rest of the colon is harbored by anaerobic bacteria 100 to 1000 times more than aerobic. It is mainly inhabited by families such as Bacteroidaceae, Prevotellaceae, Rikenellaceae, Lachnospiraceae, and Ruminococcaceae, and regarding genera the main species consist of Bacteroides, Lactobacillus, Bifidobacterium, and Clostridium. In addition the colon hosts a plethora of pathogens such as Campylobacter jejuni, Salmonella enterica, Escherichia coli (E. coli), and Bacteroides fragilis(97). In liver cirrhosis several microbiota combinations appear specific when combined to other diseases like type 2 diabetes and inflammatory bowel disease(76). Additionally, 54% of the new species that reside the gut in cirrhosis are of buccal origin. Studies are showing that as cirrhosis is progressing, significant microbiota and metabolic signatures have been associated with the first decompensation event(38) and a significant change in small intestinal environment is indicating a shift of the disease state from compensated to decompensated with portal hypertension(22). These microbiota changes in cirrhosis have produced some specific diagnostic models which were validated in the late years. These models provide both

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an approach to diagnosis and a method to evaluate new treatments. In 2014 the cirrhosis dysbiosis ratio (CDR) has been found to be a useful quantitative tool for microbiome alterations in cirrhosis. A low ratio is indicating dysbiosis(80) and it has already been used in studies evaluating treatment on microbiota with success(98). Another diagnostic model presented in 2023 is using not only microbiota features but also metabolites. The 7 microorganisms and 2 metabolites model use Subdoligranulum, Agathobacter, norank f Eubacterium coprostanoligenes group, Butyricicoccus, Lachnospiraceae UCG 004 and L-2,3-

Dihydrodipicolinate expecting to be at high levels in cirrhosis patients, and Blautia, Monoglobus, and 5-Acetamidovalerate at low levels(23). Finally a modified cirrhosis dysbiosis ratio (MDR): [Bacilli (%) + Proteobacteria (%)]/[Clostridia (%) + Bacteroidetes (%)] was able to indicate more severe states of cirrhosis in correlation to different levels of dysbiosis(47). These are tending to replace the Firmicutes/Bacteroidetes (F/B) ratio and the Microbial Dysbiosis index (MDI) which are not specific for cirrhosis(15).

Our systematic review is summarizing many microbial changes in cirrhosis but in most of them we realize a shift towards an increase in potentially pathogenic or pathogenic bacteria and a significant decrease in commensal or beneficial bacteria. In fact, bacteria that play a role as metabolizers of bile salts, as producers of protective mucus or as trasformers of significant aminoacids, lipids and sugars are constantly reducing(5, 8, 12, 63). Many studies are indicating that not only the presence of protective bacteria is reducing significantly but the potentially pathogenic outnumber them reaching the threshold of bacterial overgrowth namely > 10^3 cpu/ml(10-12, 14). This has been correlated with more advanced stages of cirrhosis especially when significant portal hypertension has developed(22). One study has showed that SIBO prevalence is significantly higher in liver cirrhosis than in non-cirrhosis. Additionally, even in non- cirrhosis when SIBO was present this was correlate with irreversible advanced liver disease(99). Another study has showed that SIBO derived by hydrogen producing bacteria is more important that SIBO from methane producers in the development of hepatic encephalopathy(100). Finally, a study published by our group found a significant correlation of SIBO with Child-Pugh score and significant motility changes in cirrhosis(16). And this was in line with another study that showed that microbiota changes are depending on small intestinal transit alterations found in cirrhosis with portal hypertension(15). The last two studies showed also that in the presence of decompensation episodes with significant portal hypertension pharmaceutical interventions that reduce SIBO like rifaximin or increase small intestinal motility like propranolol have proved efficient(16, 100). Taken together the current knowledge described in this systematic review provides evidence for clinical implications regarding specific dysbiosis scores for cirrhosis as also information for the correct use of probiotics, antibiotics and targeted therapies like motility agents as well.

This systematic review has also revealed some limitations. First as studies collected come from different areas of the world this may have influenced the heterogeneity in results and also the different dietary habits especially when healthy population is used for comparison. Additionally, many changes may be due to differences in etiology of cirrhosis or pharmacologic interventions in decompensated cirrhosis such as previous use of antibiotics and gastric antisecretory medications. Another possible limitation arises from the fact that nearly most of the studies analyzed microbiota on stool provided while the alternative of duodenal biopsies or duodenal aspirates may have proven a different biological diversity as other studies on microbiota have been indicating(101). Finally, 23 of the 73 studies included small samples of less than 50 patients that may decrease the validity of these results.

4. Conclusion

In conclusion dysbiosis of the gut microbiota is strongly associated with cirrhosis and its potential complications such as hepatic encephalopathy and HCC. Studies conducted on microbiome structure, show some heterogeneity in the results. Despite this, alpha diversity, increased abundance of pathobionts such as Enterococcus and Streptococcus and decrease in species that belong to bacteria families including Lachnospiracaceae and Oscillospiracaceae with important role in gut homeostasis

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were all linked with liver cirrhosis progression. These changes in microbiota composition affect directly and indirectly the pathophysiology of cirrhosis by many mechanisms including chronic inflammation, changes in metabolism and increased intestinal permeability. More comprehensive, large-scale studies on the role of microbiota regarding specific complications including ascites, spontaneous bacterial peritonitis and ACLF should be authorized as current knowledge lacks evidence in this matter.

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