visbiome™

HIGH POTENCY PROBIOTIC

Product Monograph Visbiome Probiotic in Liver Disease

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ExeGi PHARMA

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1. EXECUTIVE SUMMARY

Visbiome™ and Visbiome Extra Strength™ are medical foods intended for the dietary management of dysbiosis associated with pouchitis, ulcerative colitis (UC), irritable bowel syndrome (IBS), and hepatic encephalopathy (HE). Visbiome is a non-drug therapy that addresses distinct nutritional requirements, to promote microbial balance in people with IBS, UC, pouchitis and HE, which cannot be addressed by modification of the diet alone.

Visbiome contains eight (8) strains of live bacteria in concentrations of 112.5 billion (capsules) to 450 billion bacteria (powder packets) and 900 billion bacteria (Visbiome Extra Strength – powder formulation).

Alterations in the gut microbiome have been associated with a number of liver diseases (e.g. cirrhosis, non-alcoholic fatty liver disease, hepatic steatosis). Manipulation of the gut microbiome with non-systemic antibiotics, prebiotics, and probiotics has become standard of care treatment for a number of liver related conditions (such as the use of rifaxamin in the treatment of HE). In the dietary management of dysbiosis associated with HE, the formulation in Visbiome has been the subject of multiple controlled clinical studies.

Visbiome is produced by ExeGi Pharma LLC., a biotechnology company focused on the development and commercialization of live biotherapeutic and probiotic medicines. Our team leverages the growing advances in the field of microbiome science to deliver novel, clinically supported, live biotherapeutic and probiotic treatments for a wide variety of unmet medical needs.

The product website is www.visbiome.com - Company website is www.ExeGiPharma.com

2. HUMAN MICROBIOME

The human body is a complex ecological community consisting of trillions of microorganisms that exist in a beneficial and symbiotic relationship. In recent years, new technology has allowed medical science to better understand and characterize this community, which is commonly referred to as the human microbiome.

The human microbiome consists of thousands of different bacteria with significant phenotypic and genotypic diversity.. The majority of the bacteria are not harmful act to support and maintain human health, help us digest food, synthesize essential nutrients, and prevent invasion by harmful pathogenic bacteria. Studies have shown that there are actually more bacteria cells living in and on the average person than there are actual “human” cells.

Recently, the National Institute of Health (NIH) initiated the NIH Human Microbiome Project, a broad scale research effort involving hundreds of scientists and over 80 research centers whose goal is to better characterize and understand the human microbiome and its links to human health.

3. PROBIOTICS

Probiotics are live microorganisms that, when administered in an adequate amount, produce a beneficial effect to the host. Some of the main beneficial attributes of probiotics include their ability to induce changes in intestinal microbiota, improvements in the intestinal barrier, and modulation of inflammatory and immunological response. Because probiotics are an “ecologic”, non-pharmacological, and relatively cheap alternative to “classical” drugs, there has been growing interest in recent years regarding the possible usefulness of these therapeutic options in many fields of medicine.

For decades, however, the implementation of probiotics in daily clinical practice has been limited. The reasons for this restricted use include a) the existence of a high variety of probiotics with different properties and different qualities, b) the lack of high-quality trials, c) clinicians’ lack of confidence in this treatment modality, and d) regulations that differ from drugs. Nevertheless, the landscape has changed in recent years thanks to 1) the recognition of concrete properties of several specific probiotics, 2) the development of well-designed clinical trials which followed the same strict guidelines used for drugs research, and 3) the publication of positive results from these trials in high-quality peer reviewed journals. Moreover, the alarming increase in bacterial resistance as a result of the widespread use of antibiotics has created an urgent need for effective alternatives when modulation of intestinal microbiota is required.

4. HEPATIC ENCEPHALOPATHY - SUMMARY

Hepatic Encephalopathy (HE) is a disease state consisting of neurological and psychiatric manifestations found in patients with chronic liver diseases, portal hypertension, and sometimes acute liver failure. HE is a major neurological disorder and is associated with decreased survival and increased health care utilization. Although the exact cause of HE is unknown, it tends to occur in patients who have conditions impacting their liver. The liver aids in the breakdown of toxic substances within the body. However, when the liver is damaged and unable to function properly, these toxins can build up in the bloodstream and lead to neurological problems. HE is most common in patients with conditions which inhibit proper liver function or conditions in which blood is unable to properly circulate through the liver.

HE can manifest itself as a spectrum of neurocognitive disorders ranging from minimal HE (MHE), also known as covert HE, to the more serious overt HE. MHE is a subtle cognitive dysfunction that can only be diagnosed using psychometric or neurophysiological tests. Mild symptoms associated with HE include a musty or unusually sweet odor, disturbances in sleep, changes in thinking, mild confusion, mood changes, inability to concentrate, decreased dexterity, and poor judgment making abilities. Although minimal HE represents the mildest degree of HE, it is not devoid of clinical significance because it predisposes to overt HE, traffic accidents and falls, and is associated with poor prognosis and deterioration of health-related quality of life. Overt HE is characterized by altered consciousness, confusion, abnormal movements of limbs, seizures, disorientation, drowsiness, severe personality changes, slurred speech, or sluggish movement and may result in hospitalization and coma. In both MHE and overt HE, high intracerebral ammonia is postulated to have a central role in disease progression.

Treatment of HE typically focuses on modulating the gut microbiome and reducing the ammonia producing bacteria from the gut. Recommended treatments include non-absorbable disaccharides (lactulose) and minimally absorbed antibiotics. Probiotics, branched chain amino acids, and intravenous L-ornithine L-aspartate (LOLA) have also been evaluated.

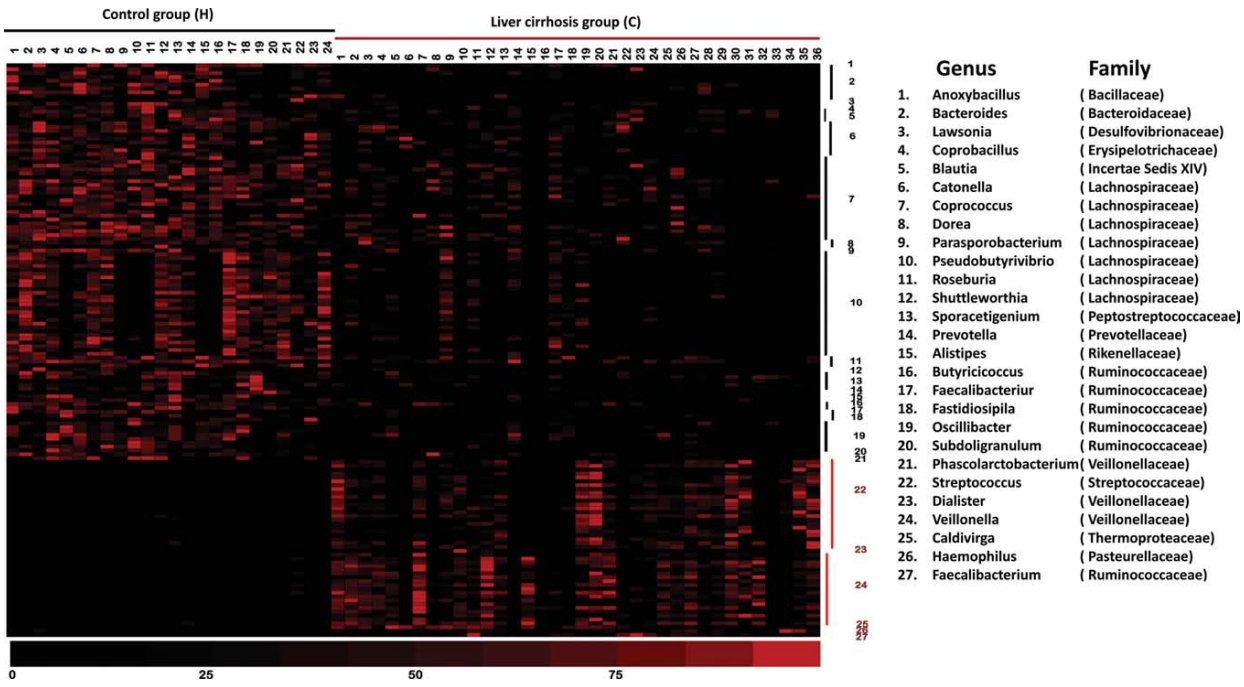
The American Association for the Study of Liver Disease (AASLD) recommends lactulose as the first choice for treatment of episodic overt HE.[34] Dosing is recommended at 25 mL of syrup every 1-2 hours until at least two soft or loose bowel movements can be maintained per day. Rifaximin is recommended as an add-on therapy for the prevention of overt HE (neomycin and metronidazole are considered alternative choice antibiotics). Lactulose is also recommended as the first choice for the prevention of recurrent episodes of HE after an initial episode, with rifaximin considered as an add-on to lactulose.

5. LIVER DISEASE AND THE MICROBIOME

5.1. Gut Bacterial Dysbiosis in Liver Disease

Numerous studies have confirmed that the microbiota of patients with liver diseases such as cirrhosis, non-alcoholic fatty liver disease (NAFLD), and steatohepatitis (NASH), is altered when compared to healthy controls.[1,2,7,22,23,24] In one study, the fecal microbiota of 36 patients with liver cirrhosis was compared to 24 healthy controls, using non-culture depend analytical techniques (i.e. 16S rRNA and real-time PCR).[1] Community–wide microbiome differences were observed with some phylum reduced and others increased in diseased vs. health controls. In the same study, gut bacterium was classified into closely related taxonomic groups called Operational Taxonomic Units (OTUs) and cirrhotic patients were compared to healthy controls. Figure 1 highlights the differences in OTUs between cirrhotic patients and controls with “good” bacterial taxonomic families. For instance, the bacterial family *Lachnospiraceae* is more abundant in the controls compared to the cirrhotic patients. Families associated with “bad,” potentially pathogenic, bacterium (e.g. *Streptococcaceae*, *Enterobacteriaceae*) are in higher abundance in cirrhotic patients.

Figure 1 - Abundance distribution of the 149 OTUs identified as key variables.



(Adapted from Chen et al. 2011 –Abundance distribution of the 149 OTUs identified as key variables. Colored squares of each row indicate the relative ratios of OTU among 60 patients)

In another study, the gut microbiome was analyzed in 25 cirrhotic patients and 10 controls using multi-tag pyrosequencing. Additional testing included comparison of patients with or without HE in inflammatory cytokine endotoxin analysis and cognitive testing. The fecal microbiota was significantly different in HE patients when compared to controls, made evident by increased levels of *Enterobacteriaceae*, *Alcaligeneceae*, and *Fusobacteriaceae* and lower *Ruminococcaceae* and *Lachnospiraceae*. Furthermore, in the cirrhosis group, specific bacterial families (*Alcaligeneceae, Porphyromonadaceae, Enterobacteriaceae*) were positively correlated with changes in cognition and inflammation in HE.[24]

Researchers found disease severity can correlate with changes in the microbiota profile. For example, through the use of 16S rRNA, researchers have found that in patients with cirrhosis the ratio of autochthonous/"good" bacteria (e.g. *Lachnospiraceae, Ruminococcaceae* and *Clostridiales*) to non-autochthonous/pathogenic "bad" bacteria (e.g. *Enterobacteriaceae* and *Streptococcaceae*) is higher both in controls and in compensated participants (no symptoms) vs decompensated (symptomatic) cirrhosis.[23]

5.2. The Role of the Gut Microbiota in Liver Disease

5.2.1 Bacterial Translocation and Inflammation in Liver Disease

There is a close relationship between the gut, the organ which harbours our intestinal microbiota, and the liver, often referred to as the "gut-liver axis". The liver receives 70% of its blood supply from the portal vein, which is connected to the stomach, pancreas, and intestine. Portal blood transports both nutrients and enteric bacteria and their by-products to the liver. These by-products include endotoxins, bacterial metabolites, and bacterial DNA which are all collectively referred to as - PAMPs: Pathogen Associated Molecular Patterns.

In normal conditions, these bacteria and PAMPs are adequately processed by the liver. However, in liver diseases, there is not only a failure in this detoxification, but there are also alterations in gut microbiota and an increase in intestinal permeability which can lead to the abnormal arrival of bacteria and PAMPs to the liver, the mesenteric lymph nodes and other extraintestinal sites. In patients with cirrhosis, and other liver diseases, these changes to the barrier integrity of the gastrointestinal tract have been well documented. This increase in intestinal permeability is often called "leaky gut", "bacterial translocation", or "pathological bacterial translocation."

Increases in intestinal permeability may be linked to a number of factors including changes to the gut microbiome and lifestyle factors, such as excessive alcohol consumptions. One factor linked to the gut microbiota concerns the production of certain "good" gut bacteria which are capable of fermenting non-digestible plant polysaccharides into short chain fatty acids such as butyrate. Butyrate is a key energy source for the cellular gut lining (colonocytes) and can help maintain mucosal integrity by supporting colonocyte and enterocyte proliferation.[22] Changes in the microbiota may reduce the production of butyrate.

The impact of bacterial translocation may cause excessive activation of hepatic immune cells, mainly Kupffer cells and hepatic stellate cells, leading to further liver damage. Translocation can also produce a systemic inflammatory response that will contribute to the immune and hemodynamic alterations involved in the development of complications of cirrhosis: HE, ascites, hepatorenal syndrome, variceal bleeding or acute-on-chronic liver failure (ACLF). Interestingly, several studies have shown that as systemic inflammation increases, so does the severity of HE.[3] For example, deterioration in neuropsychological function in cirrhosis patients tends to be more severe in those with acute inflammation or infection.[4]

5.2.2 Changes in Bile Acid Profile in Liver Disease and the Gut Microbiome

In liver diseases such as cirrhosis, altered production of primary bile acids can cause shifts in the microbiome profile. As previously noted, liver disease can lead to bacterial translocation and hepatic inflammation. Inflammation leads to changes in the synthesis of bile acids in the liver. One such effect is a potential increase in the production of cholic acid which can lead to increased *Clostridium* species and an increased production of the harmful bile acid deoxycholic acid (DCA). In addition, decreases in some bile acids entering the intestine may favour the overgrowth of other bacterial families considered pathogenic, or "bad", members of the microbiome such as *Porphyromonadaceae* and *Enterobacteriaceae*. The bile acid pool and composition may be a major regulator of the human microbiome.[5]

Changes in the microbiome may then cause a "feedback loop" which further amplifies inflammation and bile acid dysregulation. As the microbiome becomes more dysbiotic, it is likely to cause additional intestinal permeability, immune alterations and inflammation, thereby exacerbating the complications of liver disease such as HE, and further altering bile acid composition.

5.3. The Gut Microbiome and Hepatic Encephalopathy

Elevated ammonia levels are key to the pathogenesis of HE, and HE can be directly tied to microbiota metabolism. Urease-producing bacteria hydrolyse urea into carbamate and ammonia. Urease-producing bacterium are typically members of taxonomic families considered to be "bad" bacteria such as *Enterobacteriaceae, Proteus and Clostridium*.[6,25] These families may be subject to overgrowth in liver disease patients due alterations in bile acid profiles. To characterize the relative "health" of the gut microbiome in cirrhosis, investigators have even developed a ratio of "Good" vs. "Bad" bacterial taxa called the Cirrhosis Dysbiosis Ratio (CDR). A low CDR indicates a deficit of good gut bacteria.[7] Patients with decompensated cirrhosis have been shown to have a lower CDR than controls or those with compensated cirrhosis.[23]

As previously noted, the mainstays of current therapy for reducing elevated ammonia levels in HE involves the modulation of fecal flora with minimally absorbed antibiotic and lactulose. Lactulose is a non-absorbable disaccharide which has been a key treatment for HE for decades. There are likely several mechanisms of lactulose in the management of hyperammonemia. One such mechanism concerns the activity of lactulose as an energy source, or "prebiotic", for *Lactobacilli* and *Bifidobacteria* species. This selective prebiotic action may help to decrease the growth of urease-producing bacteria though competitive exclusion of available nutrients. In addition, the increased fermentation activity of *Lactobacilli* and *Bifidobacteria* species will produce lactic acid, which can acidify the fecal stream, thereby causing the protonation of ammonia into ammonium. Ammonium is poorly absorbed across the gut thereby trapping it within the fecal stream.

Minimally absorbed antibiotics are also a key component of treatment, with rifaximin (Xifaxan®) being the leading treatment. While the mechanism of antibiotics in HE is a subject of some debate, it is clear that there is a reduction in gut bacterial load which may decrease urease-producing bacterium.[6] Additional evidence shows that rifaxamin is selective for beneficial bacterial species.[8]

By modulating the gut microbiome, probiotics may play a role in the dietary management of several liver diseases by improving the intestinal barrier and modulating immune alterations and inflammatory response in the GI tract.

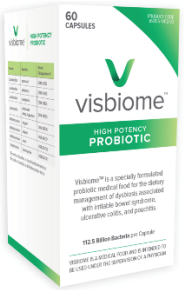
6. VISBIOME AND VISBIOME EXTRA STRENGTH PROBIOTIC

- Visbiome™ is a high potency probiotic medical food, containing eight (8) strains of live bacteria in high concentrations.
- Visbiome and Visbiome Extra Strength are medical foods intended for the dietary management of dysbiosis associated with pouchitis, ulcerative colitis (UC), irritable bowel syndrome (IBS), and hepatic encephalopathy (HE).
- Visbiome is a non-drug therapy that addresses distinct nutritional requirements, to promote microbial balance in people with IBS, UC, pouchitis, and HE that cannot be addressed by modification of the diet alone.
- Visbiome™ is a medical food intended for use under the supervision of a physician.

6.1. Visbiome Formulations

6.1.1 Visbiome Dosage Forms

VISBIOME is a powder consisting of eight (8) strains of live, lyophilized, probiotic bacteria. Visbiome is available in three (3) dosage forms:

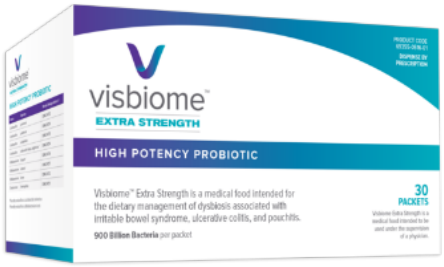


	Visbiome Regular	Visbiome Unflavored	Visbiome Capsules
Format	Box / Packets	Box / Packets	Bottle / Capsules
Units	30 Packets per box	30 Packets per box	60 Capsules per bottle
Potency	450 Billion (450×10^9) CFUs[1] per Unit	450 Billion (450×10^9) CFUs per Unit	112.5 Billion (112.5×10^9) CFUs per Unit
Inactive Ingredients	Maltose, lemon flavoring, and silicon dioxide.	Cornstarch.	Microcrystalline cellulose, stearic acid, magnesium stearate, silicon dioxide, and vegetable capsule (hydroxypropylmethylcellulose).

[1] CFU = Colony Forming Unit

6.1.2 Visbiome Extra Strength Dosage Forms – Dispensed with Prescription[2]

VISBIOME EXTRA STRENGTH is a powder consisting of eight (8) strains of live, lyophilized, probiotic bacteria. Each packet contains at least 900 billion (900 x 10^9) colony forming units (CFUs).



	Visbiome Extra Strength
Format	Box / Packets
Units	30 Packets per box
Potency	900 Billion (900 x 10^9) CFUs per Unit
Inactive Ingredients	Maltose and silicon dioxide.

6.2. Visbiome Strains

Figure 2 - Genus, Species, and Reference Number

Genus	Species	Deposit Reference DSMZ – German Collection of Microorganisms and Cell Cultures
Lactobacillus	*paracasei*	DSM 24733
Lactobacillus	*plantarum*	DSM 24730
Lactobacillus	*acidophilus*	DSM 24735
Lactobacillus	*delbrueckii* subspecies *bulgaricus**	DSM 24734
Bifidobacterium	*longum*±	DSM 24736
Bifidobacterium	*infantis*±	DSM 24737
Bifidobacterium	*breve*	DSM 24732
Streptococcus	*thermophilus*	DSM 24731

± Recently reclassified as *Bifidobacterium lactis*

* Recently reclassified as *Lactobacillus helveticus*

6.3. Dosing Dietary Management of HE

Daily Recommended Consumption in Dietary Management of Dysbiosis Associated with HE		
Product Format	**Dose**	**Total CFUs**
Capsule Dose – 112.5 CFUs	Two (2) Visbiome Capsules BID (Twice Daily)	450 Billion CFUs Per Day
Packet Dose – 450 CFUs	One (1) Visbiome Powder Packet	

[2] Not an FDA Approved Drug. A Medical Food.

6.4. Medical Food Status

The Orphan Drug Act of 1988 defines “medical food” as “a food which is formulated to be consumed or administered enterally under the supervision of a physician and which is intended for the specific dietary management of a disease or condition for which distinctive nutritional requirements, based on recognized scientific principles, are established by medical evaluation” 21 U.S.C. 360ee(b)(3). FDA regulations 21 C.F.R. 101.9(j)(8) set forth additional criteria for makers of medical food products.

VISBIOME is a medical food as defined by the Orphan Drug Act and additional FDA regulations. VISBIOME is specially formulated and processed to provide a precise mixture of certain bacterial species to the gastrointestinal tract. The gastrointestinal microflora, or “microbiome”, is important for the normal functioning of the human gastrointestinal tract.[9,10,11] The equilibrium of the gut microbiome has been shown to have a significant influence on human health and disturbances can have an adverse effect on essential GI functions.[12,13]

Patients with irritable bowel syndrome (IBS),[19,20,21,14,15] ulcerative colitis (UC), and pouchitis have documented deficiencies in luminal concentrations of lactobacilli and bifidobacteria compared with healthy individuals.[16,17,18,19,20,21] In addition, frequent and/or long-term use of antibiotic treatment in these patient populations can further exacerbate deficiencies in the microbiome. Likewise, the gut microbiome of patients with hepatic encephalopathy (HE), and associated liver conditions such as cirrhosis, have been shown to be significantly altered compared to controls.[1,2,7,22,23,24,25,26,27] For example, in patients with liver cirrhosis, differences in colonic mucosal microbiota are found in patients with cirrhosis plus HE versus those with cirrhosis without HE.
IBS, UC, pouchitis, and HE patients, thus, have distinct nutritional requirements that differ from the general population and thus require the consumption of high levels of probiotic bacteria to maintain an adequate and balanced microflora. In these patients, sufficient adjustment of the microflora cannot be achieved through modification of the normal diet.

VISBIOME is intended for those with irritable bowel syndrome (IBS), ulcerative colitis (UC), pouchitis, or hepatic encephalopathy (HE) who are receiving active and ongoing medical supervision with regular instruction on the use of medical foods.

6.5. Visbiome Safety

Probiotics have a long history of safe use, having been consumed for health benefit and as part of fermented foods for millennia.[28,29,30,31] Many bifidobacteria and lactobacilli species are normal, nonpathogenic inhabitants of the human gastrointestinal tract, oral cavity, skin, and vagina. [28,29,32,33] Documented cases of infection attributable to probiotic intake are limited to individual case reports, primarily associated with the use of probiotics in severely immunocompromised patients, but never reported for VISBIOME.

The probiotic bacteria in VISBIOME are non-pathogenic, non-toxigenic and Generally Recognized as Safe (GRAS) as food ingredients.

The probiotic formulation in VISBIOME has been the subject of clinical studies in adult and pediatric patients (ages 1.7 - 17). The most common reported adverse events are abdominal bloating and/or gas, generally reported within the first few days of probiotic consumption.

VISBIOME has been administered in clinical evaluation in daily dosages of up to 3,600 billion (360×10^{10}) colony forming units (CFUs) per day for 12 weeks.

6.6. Visbiome in Dietary Management Dysbiosis Associated with Hepatic Encephalopathy – Clinical Summary

A meta-analysis conducted by Saab et al. in 2015 analyzed 14 studies, 5 of which used the formulation contained in Visbiome. The review found that the use of probiotics in general were effective dietary agents which may improve minimal hepatic encephalopathy (MHE) and prevent progression to overt HE in patients with underlying MHE, with results similar to those with lactulose.

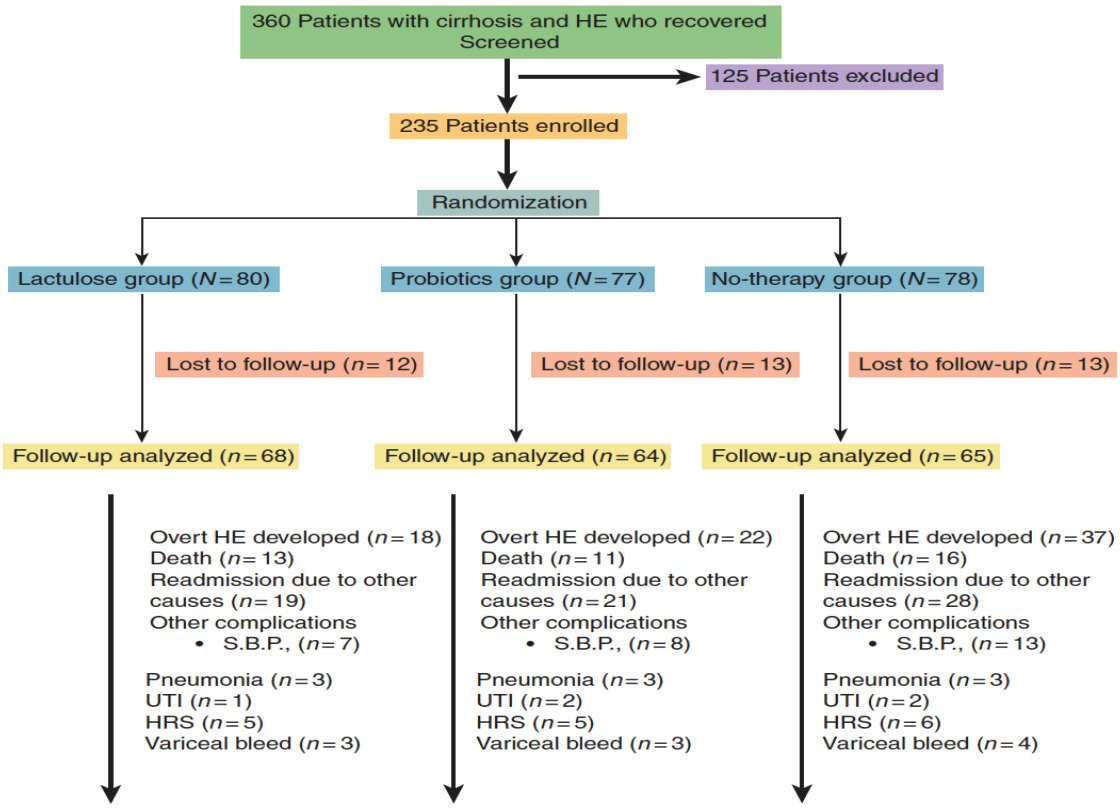
6.5.1 *Agrawal* et al. Secondary Prophylaxis of Hepatic Encephalopathy in Cirrhosis: An Open-Label, Randomized Controlled Trial. *Am J Gastroenterol.* 2012

Study Design

Agrawal *et al.* randomized 235 cirrhotic patients who had recovered from a previous episode of HE were randomized into three groups: one group received the probiotic mix for 12 months, the second group received lactulose, and the third group was the control group. HE was assessed by the West Haven Criteria. Neuropsychological performance (NP) assessment was evaluated using a battery of testing methods including; number connection tests (NCT-A,B), two figure connection tests (FTC-A,B), block design test, and the digit symbol test. In addition, psychometric tests were performed evaluating psychomotor speed, visual-spatial reasoning, and critical flicker frequency. Arterial ammonia concentration was determined immediately after psychometric testing.

Patients in the probiotic group received three capsules per day in a dosage of 112.5 billion colony forming units (each capsule contained the probiotic blend in Visbiome).

Figure 3 - Agarwal et al. Trial Design and Flow



HE Outcomes – Secondary Prophylaxis and Ammonia Levels

During the study period, 77 (39.1%) of 197 patients developed an episode of overt HE. Specifically, 22 (34.4%) in the probiotic group, 18 (34.4%) in the lactulose group, and 37 (56.9%) in the control group experienced an episode of overt HE. Lactulose therapy, as compared with no therapy, was significantly more effective in secondary prophylaxis (26.2 % vs. 56.9 % , P = 0.001). Similarly, dietary management with the probiotic, as compared with no therapy, was associated with a lower incidence in recurrence of HE (34.4 % vs. 56.9 % , P = 0.02), but no significant difference was found between lactulose and probiotic therapy (26.2 % vs. 34.4 % , P = 0.349).

Figure 4 - Agarwal et al. Probability of Developing HE (GP-N No Therapy, GP-P Probiotic, GP-L Lactulose)

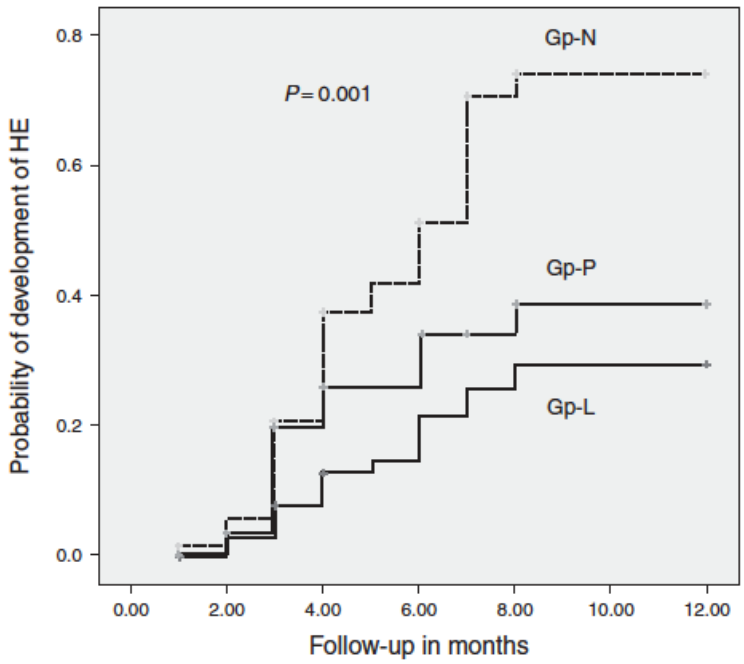


Figure 5 - Agarwal et al. Arterial ammonia level in the three groups at baseline and 3-month follow up.

	Arterial ammonia at baseline (μmol/l)	Arterial ammonia on follow-up (μmol/l)	*P* value
Lactulose group	93.2±19.0	82.97±12.9	0.03
Probiotics group	88.2±20.6	75.20±20.9	0.04
No-therapy group	89.8±18.6	85.20±16.7	0.597

Arterial ammonia levels were significantly reduced in the probiotic and lactulose groups over baseline. There was no changed in the No Therapy group.

Safety Outcomes

All patients were able to tolerate and remained compliant with lactulose therapy in lactose. Of 68 patients, 18 (26.4 %) had diarrhea, 11 (16.2 %) had abdominal bloating, and 12 (17.6 %) had distaste to lactulose. In these patients, the dose was reduced, but not stopped. In No-Therapy, constipation was reported in 14 (21.5 %) patients and was managed by dietary modification cations. In the probiotic group, 9 (14 %) patients complained of abdominal distension and 14 (21.8 %) of constipation managed with dietary advice and on-and-off use of proton pump inhibitors. None of the patients in the probiotic group developed increased frequency of stools, fever, or rash related to probiotics.

6.5.2 *Lunia et al.* Probiotics Prevent Hepatic Encephalopathy in Patients With Cirrhosis: A Randomized Controlled Trial. *Clin. Gastroenterology and Hepatology.* 2014

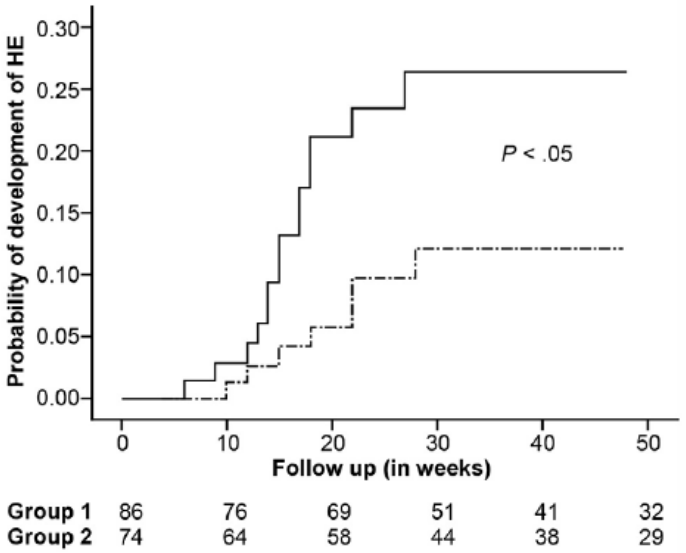
Study Design

Lunia et al. aimed to evaluate the product in the setting of primary prophylaxis of HE in patients with cirrhosis without overt HE. The primary endpoint was development of overt HE (using West Haven criteria). One hundred and sixty patients with cirrhosis and no previous HE were randomized either to a group supplemented with the probiotic mix (one 110 Billion CFU Capsule 3 times daily) for a mean period of 38.6 weeks or to a control group. Psychometric testing (using psychometric hepatic encephalopathy score – PHES), measurement of Critical Flicker Frequency Threshold and evaluation for small intestinal bacterial overgrowth (SIBO) was also performed

Outcomes

The incidence of the first HE episode was significantly lower in the probiotic group (8.8%) than in the control group (20.3%), when the probiotic was used in the dietary management of dysbiosis.

Figure 6 - Probability of developing HE – Dotted line = probiotic



The authors also observed a decrease in arterial ammonia levels after three months in the probiotic group (P=0.04) with no changes in the control group. SBIO decreased in the probiotic group vs control (P=0.006) at three months. Patients in the probiotic group also experienced statistically significant improvements in PHES (P=0.01) vs controls, which saw no improvements. Finally, the number of patients with minimal HE also decreased significantly in the probiotic group at three months (0.001) with a non-statistically significant change in the control arm.

Figure 7 - Changes in Key Parameters at 3 Months

	Group 1			Group 2		
Parameters	Baseline (n = 86)	3 months (n = 76)	*P*	Baseline (n = 74)	3 months (n = 62)	*P*
Arterial ammonia, *μmol/L*	74.3 ± 18.6	61.2 ± 15.2	.04	78.4 ± 15.6	81.3 ± 17.8	.88
SIBO, n (%)	33 (38.4)	14 (17.7)	.006	26 (35.1)	21 (33.9)	.91
OCTT, *min*	138.6 ± 22.9	112.3 ± 18.8	.05	145.6 ± 21.2	141.7 ± 21.4	.85
PHES	-5.6 ± 3.6	-2.2 ± 1.9	.01	-5.3 ± 3.1	-5.1 ± 2.9	.76
CFF, *Hz*	40.4 ± 8.8	49.9 ± 10.4	.02	41.8 ± 9.9	39.2 ± 13.1	.62
MHE, n (%)	42 (48.8)	18 (22.8)	.001	33 (44.6)	25 (40.3)	.74

SIBO – Small Intestine Bacterial Overgrowth, OCTT – orocecal transit time, PHES - psychometric hepatic encephalopathy score, CFF - Critical Flicker Frequency Threshold

Safety Outcomes

Probiotic consumption was not associated with any side effects in the study and none of the patients required discontinuation of therapy.

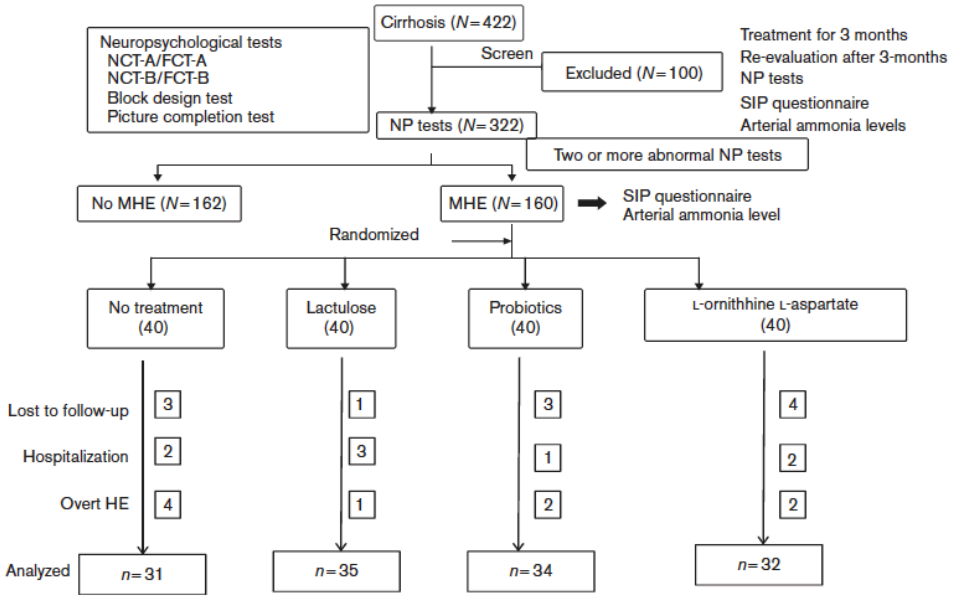
6.5.3 *Mittal et al.* A randomized controlled trial comparing lactulose, probiotics, and L-ornithine L-aspartate in treatment of minimal hepatic encephalopathy. *E. J of Gastro & Hepatol.* 2011

Study Design

Mittal et al. performed a randomized study in 160 patients with cirrhosis and minimal HE, distributed into four groups. One group received the probiotic (110 billion CFU capsule twice per day) for 3 months, the second group was treated with lactulose, the third received L-ornithine L-aspartate (LOLA) twice a day, and the fourth received LOLA three times per day. Patients were evaluated for arterial ammonia levels, neurocognitive changes, and Health Related Quality of Life (HRQoL) outcomes. Neuropsychological (NP) assessment was evaluated using a number of cognitive testing methods using number connection (NCT-A,B) figure connection tests (FTC-A,B), block design test and picture completion test.

Health Related Quality of Life (HRQoL) was measured using the Sickness Impact Profile (SIP) questionnaire, which uses a 136 metric profile of daily functioning grouped into 12 categories (e.g. sleep, home management, mobility, alertness, etc.). Patients completed the questionnaire before and after 3 months of study enrollment. At baseline, the SIP Score was comparable in all four groups. After three months there was a statistically significant reduction in the SIP scores for lactulose, probiotic, and LOLA compared to no treatment.

Figure 8 – Mittal et al. Trial Design and Flow



Trial design and flow of patients. FCT, figure connection test; HE, hepatic encephalopathy; MHE, minimal hepatic encephalopathy; NCT, number connection test; SIP, Sickness Impact Profile.

Outcomes

The authors observed similar results with the three products in terms of decrease in ammonia and improvement in psychometric tests and health-related quality of life compared to the control group. Minimal HE resolved at the end of study period in 35% of patients from the probiotic dietary management group, 47.5% in the lactulose group, 35% in the LOLA group, but only 10% in the control group (p=0.006).[39]

At baseline, all patients had at least two abnormal NP tests. At three months the number of patients with recovery from MHE (normalization of abnormal psychometry tests, i.e., <2 standard deviation of controls) were significantly more in the intervention group of lactulose (19 of 40, 47.5%), probiotics (14 of 40, 35%), and LOLA (14 of 40, 35%) compared with no treatment (four of 40, 10%) by intention-to-treat analysis, (P =0.006).

After intervention, there was a statistically significant decrease in the total SIP score (D SIP score) in the lactulose, probiotics, and LOLA groups compared with no intervention. There was also a significant decrease in total physical and psychosocial categories. Communication and eating were the only two parameters that were not statistically different with intervention compared with placebo.

6.7. Visbiome Concomitant Administration with Antibiotics/Lactulose

6.7.1 Lactulose

Lactulose is a synthetic disaccharide which is poorly absorbed by the gastrointestinal tract. Lactulose is recommended as the first choice by the AASLD Guidelines for the treatment of episodic overt HE and for the prevention of HE after the initial episode of HE.[34]

While lactulose likely has multiple mechanisms of action, evidence suggest that the primary activity is a "prebiotic" effect, which supports the growth of endogenous "beneficial" bacteria such as *Bifidobacterium* and *Lactobacillus* species.[23] These bacteria in turn help reduce ammonia levels by reducing the pH of the fecal stream and reducing the propagation of ammonia-producing bacteria (through competitive exclusion). As previously noted, fermentation of non-digestible plant polysaccharides may also produce butyrate, a key energy source for the cellular gut lining (colonocytes). This activity may help maintain mucosal integrity and prevent translocation.

Numerous studies have indicated that lactic acid-producing beneficial gut bacterial strains such as *Bifidobacterium* and *Lactobacillus* species may remain neutral, or increase, in the human GI tract with the administration of lactulose.[35,36]

Visbiome may be consumed concomitantly with lactulose as a medical food for the dietary management of dysbiosis associated with HE. Visbiome is a non-drug therapy that addresses distinct nutritional requirements, to promote microbial balance in people with HE (and other conditions) that cannot be addressed by modification of the diet alone.

6.7.2 Antibiotics

Rifaximin is a poorly absorbed antibiotic with a localized effect in the GI tract. Rifaximin is FDA approved to reduce the risk of overt HE recurrence in patients 18 years of age and older. AASLD Guidelines recommend rifaximin as an add on therapy to lactulose for the prevention of overt HE recurrence. Rifaximin is also recommended as an add-on to lactulose for the prevention of recurrent episodes of HE after a second episode of HE. Rifaximin + lactulose has been shown to reduce hospitalization rates in those with a history of overt HE. Neomycin and metrotronidazole are recognized by the AASLD guidelines as alternative antibiotic treatments. The presumed mechanism of action of antibiotics in HE is aimed at reducing ammonia producing bacteria from the gut.

One study evaluating the effect of rifaximin on the human gut microbiota has found that rifaximin (at concentrations selected to simulate an 1800 mg/day dosing regimen) did not affect the overall composition of gut microbiota; and, resulted in increased concentrations of *Bifidobacterium*, *Atopobium* and *Faecalibacterium* prausnitzii, species which may be beneficial with anti-inflammatory and immunomodulatory activities. In addition, rifaximin caused an increase in bacterium which produce short-chain fatty acid which may benefit the host intestinal mucosa by providing an energy source and promoting epithelial cell growth.[37] In a second study, the in-vitro activity of rifaximin, and comparator antibiotics, was evaluated against 536 anaerobic intestinal bacteria to determine the potential utility against different bowel flora.[38] 107 gram-positive non-spore forming rods, including dozens of *Lactobacillus* and *Bifidobacterium* species, were found to be highly resistant to rifaximin and neomycin, but susceptible to ampicillin, vancomycin and other antibiotics.

Visbiome may be consumed concomitantly with rifaximin as a medical food the dietary management of dysbiosis associated with HE. Visbiome is a non-drug therapy that addresses distinct nutritional requirements, to promote microbial balance in people with HE that cannot be addressed by modification of the diet alone.

6.8. Key Randomized Trials Summary

	Mittal, et al. 2011[39]	Agrawal, et al. 2012[40]	Lunia, et al. 2014[41]
Journal	European Journal of Gastroenterology and Hepatology	American Journal of Gastroenterology	Clinical Gastroenterology and Hepatology
Location	India	India	India
N and Patient Type	132 Minimal HE[1]	235 Previous HE	160 No Previous HE
Demographics	9 females and 123 males, mean age 42.86 years	36 females and 199 males, age range 18-70 years	64 females and 96 males; mean age 48.5 (+/- 10.5) for group 1 and mean age 49.4 (+/- 11.5) for group 2
Study Design	Randomized, Controlled	Open Label, Randomized, Controlled	Randomized, Double-blind, Placebo-controlled
Intervention Method	• 110 B caps twice daily • LOLA 3 per day 6g • Lactulose twice daily (30-60mL) • No Treatment (control)	• Three 112.5 B caps per day • Lactulose • No Therapy (control)	• Three 110 Billion bacteria capsules daily • No therapy (control)
Length of trial	3 months	12 months	Mean 38.6 weeks
End Point	• Improvement in MHE • Change in HRQoL at the end of therapy	• Development of overt HE or a follow-up of 12 months	• Development of overt HE
Results	• 3 month therapy with lactulose, probiotics, or LOLA led to improvement in MHE in 35-47.5% of patients • Minimal Hepatic Encephalopathy (MHE) improved significantly in all 3 treatments compared with no treatment • ↓ ammonia, improvement in psychometric test and quality of live in the 3 treatment groups vs. control	• ↓ recurrence of HE in Probiotic (p=0.02) and Lactulose (p=0.001) groups vs no-therapy • Lactulose group Overt HE developed in 18/58 patients • Probiotics group Overt HE developed in 22/64 patients • No-therapy group: Overt HE developed in 37/65 patients	• ↓ HE incidence, ↓ ammonia, ↓ SIBO [4], improvement in psychometric test • A significant number of patients in the experimental group achieved clinical remission. Clinical response was seen in the experimental group as made evidence by a lower observed stool weight than the control group.

7. TABLE OF FIGURES

8. REFERENCES

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