

# De Simone Formulation and Liver Diseases Monograph



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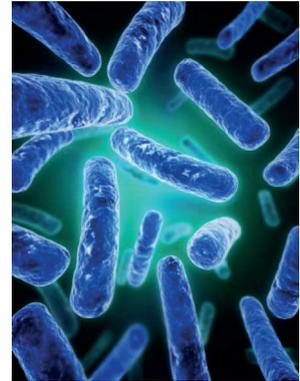
Scientific information about  
The De Simone Formulation

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## 1. Probiotics

Probiotics are live microorganisms that, when administered in an adequate amount, produce a beneficial effect to the host by changing the intestinal microbiota, improving the intestinal barrier, and modulating any inflammatory response. As probiotics are an ecological, non-pharmacological, and relatively cheap alternative to classical medications, there is a recent growing interest regarding the possible use of these therapeutic options in many fields of medicine.

However, for decades, the implementation of probiotics in daily clinical practice has been limited. There are several reasons for their restricted use, including the large variety of probiotics with different properties and qualities and the lack of high quality clinical trials and prescriber confidence in these treatments. The regulations for their use also differ from those of other investigational medicinal products. Nevertheless, this scenario has been changing in recent years, thanks to the publication of results from well-designed clinical trials in peer-reviewed journals, showing that several specific probiotics have beneficial properties. Moreover, the alarming increase in bacterial resistance due to the widespread use of antibiotics has created an urgent need for effective alternatives when modulation of the intestinal microbiota is required.



## 2. Pathophysiology of liver diseases

Pathological bacterial translocation can produce a systemic inflammatory response that will contribute to the immune and haemodynamic alterations involved in the development of complications of cirrhosis<sup>(1)</sup>. These complications include infections, ascites, hepatorenal syndrome, variceal bleeding, or acute-on-chronic liver failure (ACLF). Another complication is hepatic encephalopathy, where the excessive production and absorption of ammonia in the gut plays a synergistic role with cerebral inflammation<sup>(2)</sup>.



Therefore, probiotics can be helpful as part of a global therapeutic approach to several liver diseases and prevent complications from cirrhosis by modulating gut microbiota<sup>(3)</sup>, improving the intestinal barrier<sup>(4)</sup>, and modulating immune alterations and the inflammatory response<sup>(5)</sup>.

## 3. The De Simone Formulation

The De Simone Formulation (DSF) is a specific multispecies probiotic combination that consists of a mixture of eight strains of bacteria: *Streptococcus thermophilus* DSM 24731® / NCIMB 30438, *Bifidobacterium breve* DSM 24732® / NCIMB 30441, *Bifidobacterium longum* DSM 24736® / NCIMB 30435\*, *Bifidobacterium infantis* DSM 24737® / NCIMB 30436\*, *Lactobacillus acidophilus* DSM 24735® / NCIMB 30442, *Lactobacillus plantarum* DSM 24730® / NCIMB 30437, *Lactobacillus paracasei* DSM 24733® / NCIMB 30439, *Lactobacillus delbrueckii* subsp. *bulgaricus* DSM 24734® / NCIMB 30440\*\*. The combination of multispecies probiotics can provide a greater effect than a single strain due to their potentially synergistic or additive effects on the pathways that require modification. Here, we review the most recent and representative experimental and clinical studies that have evaluated this probiotic combination in liver diseases.

\* Reclassified as *B. lactis*

\*\* Reclassified as *L. helveticus*

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## 4. Experimental studies

Most experimental studies evaluating the De Simone Formulation in liver diseases have been conducted in models of non-alcoholic fatty liver disease (NAFLD). In these models, intestinal overgrowth develops, and an overexpression of tumour necrosis factor alpha (TNFα)<sup>(6)</sup>.

The DSF has been reported to increase hepatic peroxisome proliferator-activated receptor alpha (PPARα) expression<sup>(7)</sup>, to decrease the level of the inflammatory cytokine tumour necrosis factor alpha (TNFα)<sup>(8)</sup> and to decrease the activity of nuclear factor (NF-κB)<sup>(7)</sup>, c-Jun N-terminal kinase (JNK)<sup>(6)</sup>, metalloproteinase (MMP-2) and MMP-9, in the liver<sup>(7)</sup>.

Inducible nitric oxide synthetase (iNOS) and cyclooxygenase-2 (COX-2) expression, and hepatic natural killer T cell (NKT) depletion are involved in the pathophysiology of NAFLD. Pre-clinical studies in rodent models of NAFLD and cirrhosis have shown that this probiotic mix decreases insulin resistance, steatosis<sup>(9)</sup>, liver inflammation<sup>(3,10)</sup> and fibrosis<sup>(11)</sup>.

In a rat model of alcoholic intestinal injury evaluating the effect of the DSF on the intestinal barrier, Chang et al. observed an increase in the intestinal expression of tight junction proteins zonula occludens (ZO)-1 and occludin, and a decrease in plasma endotoxin and serum TNF $\alpha$ . These results suggest a protective effect of the probiotic mix on the intestinal barrier, reducing bacterial translocation and the systemic inflammatory response<sup>(8)</sup>.

Esposito et al. fed rats a high-fat liquid diet (HLD), which induced liver lipid peroxidation, TNF $\alpha$  production, protein S-nitrosylation, iNOS, COX-2 expression, and metalloproteinase (MMP) activity. However, in the group that received the DSF, levels of TNF $\alpha$ , MMP-2 and MMP-9 activities, and the expression of iNOS and COX-2 in the liver were significantly lower than in the group that did not receive probiotic. Suggesting that the DSF could limit oxidative and inflammatory liver damage<sup>(7)</sup>.

Studies by Liang et al. have shown that hepatic natural killer T (NKT) cells play a significant role in the pathogenesis of NAFLD. By inducing NAFLD in mice with a high-fat lipid diet and evaluating the response of high- and low-dose probiotic mix: their results revealed that alterations in gut flora have profound effects on hepatic NKT cells and steatosis, which are both strain-specific and dose-dependent<sup>(12)</sup>. Similar results were found by Ma et al. where oral probiotic treatment in mice significantly improved the high-fat, diet-induced hepatic NKT cell depletion, insulin resistance and hepatic steatosis<sup>(9)</sup>.

In the mouse model used by Velayudham et al. the more advanced stage of NAFLD, non-alcoholic steatohepatitis (NASH), was induced by a methionine-choline-deficient diet. The DSF modulated liver fibrosis but did not protect from inflammation and steatosis in NASH<sup>(11)</sup>.

Li et al. treated NAFLD mice with the probiotic mix or anti-TNF antibodies. While the DSF did not decrease the expression of TNF $\alpha$  messenger RNA, both the probiotic and anti-TNF $\alpha$  antibody treatment reduced the activity of JNK, a TNF-regulated kinase that promotes insulin resistance, and decreased the DNA binding activity of NF- $\kappa$ B<sup>(6)</sup>.

Rashid et al. evaluated the probiotic mix in an experimental rat model of portal hypertension, achieved by common bile duct ligation (CBDL). The authors observed that probiotic ingestion prevents endothelial dysfunction in the mesenteric artery of CBDL rats. This effect was associated with improved vascular oxidative stress, likely due to the reduction in bacterial translocation and the local angiotensin system<sup>(13)</sup>.

In a recent study of mice with CBDL, D'Mello et al. found that the probiotic mix attenuated sickness behaviour development, which was associated with reductions in cerebral microglial activation, monocyte infiltration, and paralleled with a decreased circulation of TNF $\alpha$ . The authors state that DSF can improve abnormal behaviours in patients with systemic inflammatory conditions<sup>(14)</sup>.

Further, Rackayová et al. published data from a rat model of chronic liver disease induced hepatic encephalopathy via bile duct ligation<sup>(15)</sup>. The ultra-high field *in vivo* 1H magnetic resonance spectroscopy (<sup>1</sup>H MRS) was combined with behavioural tests to determine whether the probiotic mix affected locomotor activity and neurometabolic changes. Prophylactic administration of the probiotic mix led to improved performance in behavioural tests and the neurometabolic profile, reducing or delaying disease progression. Treated animals displayed a significantly lower increase in brain glutamine (Gln), a milder decrease in myoinositol (mIns), and a smaller decrease in glutamate (Glu) than in untreated animals.

Other pre-clinical studies have evaluated the effect of probiotics rodent models of cirrhosis. In a rat model of carbon tetrachloride (CCI)-induced cirrhosis, Sánchez et al. showed that the DSF decreased ascites formation, bacterial translocation, and serum TNF $\alpha$  levels. Interestingly, these effects were associated with an increase in the intestinal expression of occludin and a decrease in ileal oxidative damage, evaluated by malondialdehyde (MDA) levels. No significant changes were observed in gut microbiota. Therefore, these data suggest an improvement in the intestinal barrier explains the positive effect of this bacterial blend<sup>(5)</sup>.

Zhang et al. disrupted the intestinal homeostasis in a rat model of hepatocarcinogenesis. The subsequent administration of DSF dramatically reduced enteric dysbacteriosis, improved intestinal inflammation, and decreased liver tumour growth. The DSF inhibited the translocation of endotoxin, and cytokine production was skewed towards reducing the tumorigenic inflammation in the liver. Their data highlights the importance of gut homeostasis in the pathogenesis of hepatocellular carcinoma (HCC)<sup>(10)</sup>.

The DSF has proven to be safe and well-tolerated, and therefore the clinical implications are potentially far-reaching.

## 5. Clinical studies

### 5.1 Hepatic encephalopathy

Most of the evidence supporting the efficacy of the DSF has been in the study of hepatic encephalopathy (HE). Randomised clinical studies with a large number of participants have evaluated the DSF in minimal HE (MHE), in the prevention of HE recurrence, and in the primary prophylaxis of HE settings.

MHE is a subtle cognitive dysfunction that can only be diagnosed using psychometric or neurophysiological tests. Although MHE represents the mildest degree of HE, it remains clinically significant. It represents a substantial burden on healthcare resources<sup>(16)</sup> and predisposes individuals to OHE, traffic accidents and falls<sup>(17)</sup>. It is associated with poor prognosis<sup>(18)</sup> and a deterioration in health-related quality of life<sup>(19)</sup>.

A meta-analysis by Saab et al. analysed data from 14 studies (n=1,152), five of which had the specific combination of strains. They confirmed probiotics effectively decreased hospitalisation rates, improved MHE and prevented progression to overt HE (OHE) in patients with underlying MHE, with results similar to those with lactulose<sup>(20)</sup>.

Roman et al. evaluated the effect of DSF on cognitive function, risk of falls (Timed Up and Go [TUG] test, gait speed, and incidence of falls), and inflammatory response in patients with cirrhosis and cognitive dysfunction, with or without previous falls (n=36). Patients received either DSF or placebo (n=18) twice daily for 12 weeks. An improvement in the Psychometric Hepatic Encephalopathy Score (PHES) (p=0.006), TUG time (p=0.015) and gait speed (p=0.02), was seen in patients treated with DSF, with a trend towards a lower incidence of falls (0% vs 22.2%), compared to those who received placebo. A decrease in inflammatory markers was also seen in the DSF group<sup>(17)</sup>.

Mittal et al. published results from a study of 160 patients with cirrhosis and minimal HE, randomised into four groups. One group received the DSF for three months, the second group was treated with lactulose, the third received L-ornithine L-aspartate (LOLA), and the fourth was a control group. The authors observed a similar decrease in ammonia, improvement in psychometric tests, and health-related quality of life, between the three treatment groups, compared to the control group. At the end of the study period, minimal HE resolved in 35 per cent of the patients from the DSF group, 47.5 per cent in the lactulose group, 35 per cent in the LOLA group, but only 10 per cent in the control group (p=0.006)<sup>(21)</sup>.

Mouli et al. published results following a study of patients with MHE, randomised to receive either the DSF (n=33) or lactulose (n=40) for a two-month treatment period. MHE improved in 62.5 per cent of patients taking lactulose and 69.7 per cent of those taking the probiotic mix. For those whose MHE showed improvement, serum ammonia decreased comparably in both treatment groups but not in those who had no improvement. These results confirmed that the probiotic mix was non-inferior to lactulose<sup>(22)</sup>.

A double-blind, randomised trial on patients with cirrhosis and previous HE patients published by Dhiman et al. showed a trend in reducing the development of breakthrough HE in those who received the DSF for six months (34.8%), compared to those who received placebo (51.6%). Fewer patients in the probiotic group were hospitalised due to HE (19.7%), compared to the placebo group (42.2%), or due to complications of cirrhosis (24.2% vs 45.3%). Between baseline and six months, end-stage liver disease scores improved significantly in those in the probiotic group, but not those on placebo<sup>(23)</sup>.

Lunia et al. published results from a randomised controlled trial of patients with cirrhosis without overt HE to evaluate the efficacy of the DSF as primary prophylaxis of HE. Patients received either the probiotic mix (n=86) for a mean period of 38.6 weeks ( $\pm$  8.80) or placebo (n=74) for a mean period of 40.3 weeks ( $\pm$  9.80). Seven patients in the probiotic group developed an HE episode, compared to 14 in the placebo group. Probiotic administration significantly reduced arterial ammonia, small intestine bacterial overgrowth (SIBO), and orocecal transit time (OCTT), increased psychometric HE scores, and increased critical flicker fusion (CFF) thresholds<sup>(18)</sup>.

Agrawal et al. conducted a secondary prophylaxis study on 235 patients who had suffered from HE previously but recovered. Patients were randomised to receive either lactulose (n=80), DSF (n=77), or no therapy (n=78). The primary endpoint was the development of overt HE, or a follow-up of 12 months. The recurrence of overt HE occurred in 18 patients administered lactulose, 22 administered probiotics, and 37 without treatment. Significantly fewer patients who received probiotics or lactulose had a recurrence of HE compared to those who had no treatment<sup>(24)</sup>.

Marlicz et al. published results from a short-term clinical study of 20 patients with compensated and decompensated liver cirrhosis and ten healthy controls who received the probiotic mix for 28 days. Plasma levels of interleukin 6 (IL-6), vascular endothelial growth factor (VEGF), plasminogen activator inhibitor (PAI), macrophage inflammatory protein 3 $\alpha$  (MIP-3 $\alpha$ /CCL20), monocyte chemotactic protein-1 $\alpha$  (MCP-

1/CCL2), human myeloperoxidase (MPO), nitric oxide (NO), prostaglandins, thromboxane (TXB<sub>2</sub>) and big-endothelin, were measured. The incidence of HE was assessed by critical flicker frequency<sup>(25)</sup>. They found that the stage of liver cirrhosis correlated with an increase in IL-6, MIP-3 $\alpha$ /CCL20, MPO, NO, TXB<sub>2</sub>, and big-endothelin. Although the grade of encephalopathy remained unchanged in patients with compensated and decompensated cirrhosis, the probiotic improved several clinical and biochemical parameters.

None of these studies reported relevant side effects attributable to the DSF.

The most relevant trials are summarised in Table 1.

Ref.	N° and type of patients	Intervention	Duration	Results
<b>Mittal 2011</b>	160 MHE	Comparator DSF, lactulose, LOLA, control	3 months	↓ ammonia, improvement in psychometric tests and quality of life, between the three treatment groups, vs control. MHE resolved in 35% DSF, 47.5% lactulose, 35% LOLA, 10 % controls.
<b>Agrawal 2012</b>	235 Previous HE	DSF, lactulose, control	12 months	↓ HE recurrence in DSF and lactulose vs control.
<b>Dhiman 2014</b>	130 Previous HE	DSF vs placebo	6 months	↓ hospitalization for HE, improvement in psychometric tests, health-related quality of life, liver function and inflammatory response.
<b>Lunia 2014</b>	160 No previous HE	DSF vs placebo	mean 38.6 weeks	↓ HE incidence, ↓ ammonia, SIBO, and OCTT. Improvement in psychometric tests.
<b>Mouli 2015</b>	120 Minimal HE	DSF vs lactulose	2 months	Similar improvement in psychometric tests
<b>Marlicz 2016</b>	20 Liver cirrhosis	DSF	28 days	Improvement in clinical and biochemical parameters.
<b>Roman 2019</b>	36 cirrhosis + HE $\pm$ previous falls	DSF vs placebo	12 weeks	Improved risk of falls, cognitive function, inflammatory response.

*Table 1. Randomised clinical trials evaluating the De Simone Formulation (DSF) in patients with cirrhosis and hepatic encephalopathy.*

## 5.2 Portal hypertension

Considering the role of bacterial translocation and the proinflammatory state in the pathophysiology of haemodynamic alterations in cirrhosis, another potential target for probiotics is to decrease portal pressure to prevent complications such as variceal bleeding, ascites and hepatorenal syndrome.

Several authors have investigated the effect of the probiotic combination on portal pressure in patients with cirrhosis and ascites. Rincón et al. performed hepatic and systemic haemodynamic evaluations at baseline and after six weeks of probiotic treatment (n=12). They reported a reduction in hepatic venous pressure gradient (HVPG, p<0.001) by at least 10 per cent in eight patients (67%) and decreases in cardiac index (p<0.01), and heart rate (p<0.01), and increases in systemic vascular resistance (p<0.05), mean arterial pressure (p<0.06), and an increase in serum sodium in most patients (p<0.01)<sup>(26)</sup>.

Tandon et al. evaluated eight patients with compensated or very early decompensated cirrhosis and hepatic venous gradient (HVPG) >10 mmHg, who received the DSF for two months. The HVPG, intestinal permeability, endotoxin, TNF $\alpha$ , IL-6, IL-8, renin and aldosterone were measured. While there was no change in the HVPG or intestinal permeability, there was a trend in the reduction of plasma endotoxin (p<0.09), an unexplained mild but significant increase in TNF-a (p<0.02), and a substantial decrease in plasma aldosterone (p<0.03), which suggest possible beneficial effects of probiotics in this patient group<sup>(27)</sup>.

Jayakumar et al. conducted a randomised, double-blind, placebo-controlled trial on patients with

decompensated cirrhosis and hepatic venous gradient (HVPG) >10 mmHg. Data from 15 patients showed that those receiving the DSF had a sharper decrease in HVPG (-11.6%) than those who received placebo (+2.8%), although this was not statistically significant in either group. No significant changes were seen in the biochemical parameters measured, except for plasma aldosterone levels in patients who received the probiotic mix<sup>(28)</sup>.

A limitation of the above studies is the small number of patients, and all authors mention the need for future studies.

In a larger double-blind, placebo-controlled trial conducted by Gupta et al. 94 patients with cirrhosis and oesophageal varices, without previous variceal bleeding, were randomised to receive placebo, norfloxacin, or probiotics DSF for two months. All patients received propranolol as the standard prophylaxis for variceal bleeding. The mean fall in HVPG was more remarkable with adjunctive probiotics (3.7mm Hg vs 2.1mm Hg,  $p=0.061$ ) or adjunctive antibiotics (3.4mm Hg) than with placebo. The haemodynamic response rate was higher in patients who received probiotics (58%) or norfloxacin (54%), compared to those who received placebo (31%) ( $p=0.046$ ). Serum TNF $\alpha$  decreased in both treatment groups but not in the placebo group<sup>(29)</sup>.

• Prevention of the first episode of hepatic encephalopathy
• Prevention of recurrence of hepatic encephalopathy
• Improvement in psychometric tests in patients with minimal hepatic encephalopathy
• Decrease in the rates of hospitalisation due to hepatic encephalopathy
• Improvement in liver function tests
• Improvement in health-related quality of life
• Decrease in ammonia
• Modulation of inflammatory response
• Decrease in portal pressure

*Table 2. Summary of the clinical observations in patients with cirrhosis following probiotic supplementation*

### 5.3 Obesity and non-alcoholic fatty liver disease (NAFLD)

In a non-controlled study by Loguercio et al. patients with several chronic liver diseases, including NAFLD, were treated with the DSF. Routine liver tests, plasma TNF $\alpha$ , IL-6, IL-10, malondialdehyde (MDA), 4-hydroxynonenal (4-HNE), S-nitrosothiols (S-NO), were evaluated on days -30, 0, 90, and 120. In patients with NAFLD, a decrease in serum aminotransferases, oxidative damage and nitric oxide was observed<sup>(30)</sup>.

Duseja et al. conducted a double-blind, proof of concept study, in 30 adult patients with NAFLD. In addition to all patients being advised on lifestyle modifications each were randomised to receive either DSF or placebo, for one year. In comparison to baseline, hepatocyte ballooning, lobular inflammation, NAFLD activity score (NAS), levels of ALT, leptin, TNF $\alpha$ , and endotoxins were seen in the DSF group at one year, compared to the placebo group<sup>(31)</sup>.

A study in obese children with non-alcoholic steatohepatitis ( $n=44$ ) by Alisi et al. evaluated changes in fatty liver (FL) severity by ultrasonography following four months of DSF supplementation. At the end of the study, the probability that children supplemented with the probiotic had no FL was 21 per cent, light FL 70 per cent, moderate FL nine per cent, or zero in the case of severe FL. Corresponding values for the placebo were zero for no FL, seven per cent for light FL, 76 per cent for moderate FL, and 17 per cent for severe FL ( $p< 0.001$ ). A statistically significant decrease in body mass index was also seen in children supplemented with the probiotic mix<sup>(32)</sup>.

Obesity often leads to severe cardiovascular diseases and diabetes, representing a heavy economic burden.

Chong et al. conducted a double-blinded, placebo-controlled, proof-of-concept study in 35 patients who had NAFLD for a short duration (average age  $57\pm 8$  years, BMI  $32.6\pm 5.0\text{kg/m}^2$ ). Measurements of endothelial function, oxidative stress, inflammation, insulin resistance, and liver injury were taken before and after

supplementation. Their results support an association between endothelial dysfunction and inflammation in patients with NAFLD and suggest that NAFLD is linked with insulin resistance<sup>(33)</sup>.

Rajkumar et al. conducted a placebo-controlled study in overweight adults aged 40-60 years (n=60). The patients were equally randomised into four groups receiving either placebo, omega-3 fatty acid, DSF, or both omega-3 fatty acid and DSF, for six weeks. Patients who received the DSF had a significant reduction in total cholesterol, triglyceride, low-density lipoprotein (LDL), very low-density lipoprotein (VLDL) and increased high-density lipoprotein (HDL) ( $p < 0.05$ ). The DSF improved insulin sensitivity, decreased high-sensitivity C-reactive protein (hsCRP), and favourably affected the gut microbiota composition. The addition of omega-3 fatty acid with the DSF had a more pronounced effect on HDL, insulin sensitivity and an improvement in the inflammatory marker hsCRP<sup>(34)</sup>.

## 6. Conclusions

The first mention of the specific mix of bacteria in the De Simone Formulation (DSF) was in the 'Recommendations for Probiotic Use - 2015 Update: Proceedings and Consensus Opinion'<sup>(35)</sup>, following a workshop organised by Yale and Harvard Universities. Recommendations for the management of liver conditions were added in this update for the first time.

In particular, level A evidence was recognised in hepatic encephalopathy. The evidence built over the years has allowed the De Simone Formulation to be recognised as a medical food for HE in the US. Level I evidence for Liver Diseases was confirmed in the Clinical Guide to Probiotic Products available in the US 2021<sup>(36)</sup>.

Indeed, several randomised clinical trials, including a large number of cirrhosis patients, have demonstrated the possible application of this specific probiotic combination in the DSF in primary and secondary prevention of HE and MHE. These trials also revealed an improvement in liver function tests and a decrease in the rates of hospitalisation. There is a potential role for DSF to decrease portal pressure and prevent related complications in patients with cirrhosis. One central positive aspect evident from the use of DSF in these indications is that it improves intestinal permeability, avoiding or reducing bacterial translocation and reducing the inflammation of the liver. DSF could also be of benefit to patients with NAFLD and alcoholic liver disease. A critical target should be tackling obesity in children, which has become the most common cause of chronic liver disease in children and is a significant burden of healthcare systems globally. The severity of hepatic steatosis is affected by intestinal permeability and intestinal bacterial overgrowth. There is a difference in the distinct composition of the gut microbiome among children and adolescents with non-alcoholic steatohepatitis (NASH), obese children without NASH, and healthy individuals. Modulation of the intestinal microbiota may offer an important therapeutic target for NAFLD, as suggested by Miloh et al.<sup>(37)</sup>.

Obesity in adults leads to a high risk of metabolic syndrome. Using this specific probiotic combination to improve the lipid profile, insulin sensitivity, and inflammatory responses may help reduce the risks of heart disease, diabetes, and stroke, in a healthy overweight population<sup>(34)</sup>.

Future research targets include using the probiotic mix to prevent hepatocellular carcinoma<sup>(10)</sup> and their prophylactic use of bacterial infections that avoid the development of bacterial resistance observed with antibiotic prophylaxis<sup>(38)</sup>. Microbiota-targeted biomarkers may be a powerful tool for the diagnosis of different diseases<sup>(39)</sup>.

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A member of the American College of Gastroenterology, Claudio De Simone is a retired Professor of Infectious Diseases of the University of L'Aquila (Italy), specialized in Gastroenterology, Allergology and Clinical Immunology. His interest in the human microbiota goes back more than 25 years when the understanding of the importance of the role of the intestinal flora was in its infancy.



# The Letter of probiotics

THE BLOG OF A PIONEER IN PROBIOTICS

INFORMATION FOR HEALTHCARE PROFESSIONALS ONLY

The Letter of probiotics ([probiotixx.info](http://probiotixx.info)) is the blog of Prof. Claudio De Simone, a pioneer in the intestinal microbiota and inventor of a probiotic mixture (8 strains, 450 billion bacteria) known as the De Simone Formulation.

The information posted on this website is dedicated to healthcare professionals.

Important information brochures and updates on the action of probiotics and on the De Simone Formulation (studies and scientific publications on IBD, IBS, liver diseases, autism etc.) are available subject to registration in the Healthcare Professional section of Prof. De Simone's blog:

LIVER DISEASES IBD AUTISM IBS

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