Our Probiotic Research

ADM®

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Foreword

There has certainly been an influx of probiotic products on the market over the last 5 – 10 years and with over 21,000 research articles on probiotics listed on PubMed, we undoubtedly seem to be in a gut era.

These probiotic products take on a range of forms from yoghurt drinks, oat based products and chocolate to dietary supplements presented in capsules, tablets, liquid or powder form. With all of these different variations it is hard to know which product to choose without knowing more in depth information about the product and the company behind it.

Probiotic trials now being published are of good quality and have convincing results with much of the work being done in digestive disorders, immune response and pathogen inhibition. However, there is other work that also looks promising in the field of (amongst others) cancer prevention, obesity and autism.

This booklet provides an insight into ADM Protexin (manufacturers of healthcare products Bio-Kult & Lepicol) and an update on research carried out to date.

About us

ADM Protexin manufacture probiotic supplements which are sold in over 80 countries worldwide. Manufacturing is at our purpose built facility in Somerset, South West, UK. Quality is of paramount importance, with a dedicated quality department and accreditations including cGMP and ISO 9001:2015, you can be sure each product is manufactured to the highest standard. All of our products are tested to ensure that they meet label claims using independent, UKAS accredited laboratories.

To ensure that we continue to produce innovative, research based products we work with leading researchers at universities and institutes to ensure that we are always at the forefront of research.

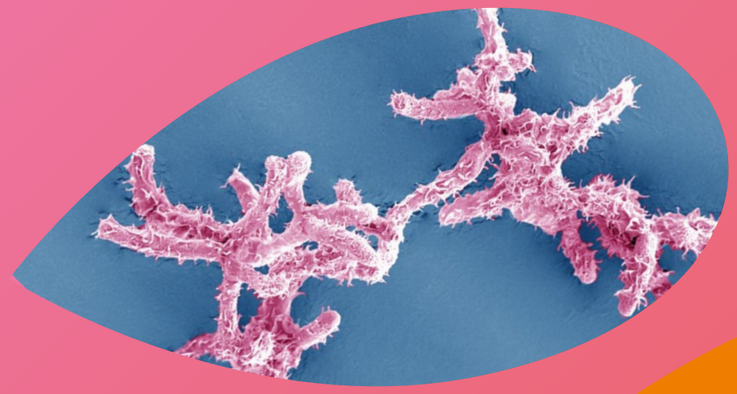
Our Protexin strains

Our Protexin probiotic species are included in the European Qualified Presumption of Safety (QPS) list. The QPS lists were compiled by the European Food Standards Agency (EFSA) to assess the compiled evidence and confirm the safety and nomenclature of the bacteria used.

Our probiotic strains have an original strain lodged at a leading UK culture collection bank known as the National Collection of Industrial, food & Marine Bacteria (NCIMB) to ensure there is no genetic shift. Here, the master cell bank, composed of freeze-dried ampoules, is stored between 2°C to 8°C. Strain purity is confirmed by the absence of bacteriological contamination. From the Master Cell Bank, the Working Cell Bank cryovials are prepared and stored at -80°C.

Strain	Culture Collection number
Bacillus subtilis PXN® 21®	NCIMB 30223
Lactobacillus casei PXN® 37®	NCIMB 30185
Lactobacillus rhamnosus PXN® 54®	NCIMB 30188
Streptococcus thermophilus PXN® 66®	NCIMB 30189
Lactobacillus acidophilus PXN® 35®	NCIMB 30184
Bifidobacterium breve PXN® 25®	NCIMB 30180
Bifidobacterium longum PXN® 30®	NCIMB 30182
Lactobacillus bulgaricus PXN® 39®	NCIMB 30186
Bifidobacterium infantis PXN® 27®	NCIMB 30181
Bifidobacterium bifidum PXN® 23®	NCIMB 30179
Lactococcus lactis subsp *lactis* PXN® 63®	NCIMB 30222
Lactobacillus plantarum PXN® 47®	NCIMB 30187
Lactobacillus helveticus PXN® 45®	NCIMB 30224
Lactobacillus salivarius PXN® 57®	NCIMB 30225
Lactobacillus fermentum PXN® 44®	NCIMB 30226

NCIMB – National Collection of Industrial, Food & Marine Bacteria.



Pre-clinical Data

Our Protexin strains

Antimicrobial activity of Protexin (PXN) strains

Protexin probiotics inhibit pathogens and have good cell adhesion properties

Objective

To assess the ability of Protexin probiotic strains to inhibit five common pathogens as well as haemolytic and hydrophobicity properties of these strains.

Methods

The inhibition activity was measured using agar spot method to ascertain the effect and well diffusion assays were used to look at the mechanism of action. The pathogen was spread onto agar plates and then the supernatant of a probiotic was pH neutralised with a buffer. If the supernatant had no effect then it would be assumed that acid is likely to be the cause of the inhibition.

Haemolytic activity was measured by using Columbia Agar with 5% Oxoid. Strains that produced green-hued zones around the spots of probiotics (α-haemolysis) or did not produce any effect on the blood plates (γ-haemolysis) were considered non haemolytic. Strains displaying blood lysis zones around the spots were classified as haemolytic (β-haemolysis).

Hydrophobicity of the Protexin strains was evaluated by the microbial adhesion to hexadecane (MATH) assay. Strains adhering well to hydrocarbons were considered to be hydrophobic and strains adhering poorly were considered to be hydrophilic.

Results

Inhibition effect of Protexin strains on selected pathogens

	Pathogen strain													
	S. typhimurium			*S. aureus*			*E. coli*			*E. faecalis*			*C. difficile*	
	8h	24h	48h	8h	24h	48h	8h	24h	48h	8h	24h	48h	24h	48h
L. acidophilus PXN 35	+++	+++	+++	+++	++	++	+++	++	++	+++	++	++	+++	+++
L. rhamnosus PXN 54	+++	++	++	++	++	++	++	++	+	+++	+	++	++	++
L. plantarum PXN 47	+++	+++	+++	+++	++	++	+++	++	++	+++	++	++	+++	++
L. bulgaricus PXN 39	+++	++	++	++	++	+	+++	++	++	++	++	++	++	+
L. casei PXN 37	+++	+++	+++	+++	++	++	+++	++	++	++	++	++	+++	++
L. lactis PXN 63	+++	++	++	++	++	+	+++	++	++	++	+	+	++	+
L. salivarius PXN 57	+++	+++	+++	+++	++	++	+++	+++	+++	+++	++	++	+++	+++
L. fermentum PXN 44	+++	++	+	+++	+	-	+++	++	++	++	+	-	++	++
L. helveticus PXN 45	+++	++	++	+++	++	++	+++	++	++	++	+	+	+++	++
B. bifidum PXN 23	++	++	++	++-	++	++	++	++	++	++	++	++	-	-
B. breve PXN 25	+++	++	++	++	+	+	++	++	++	++	+	+	+++	++
B.infantis PXN 27	+	-	-	+	-	-	++	-	-	±	-	-	-	-
B.longum PXN 30	++	++	++	++	++	++	++	++	++	++	++	++	-	-
S. thermophilus PXN 66	++	±	±	++	±	±	++	±	±	++	-	-	-	-

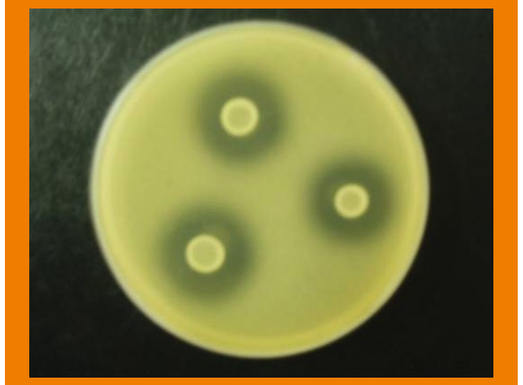
Key:
(±) < 1 cm of inhibition but no clear halo
(-) no inhibition
(+) zone of inhibition between 0.5 – 1 cm
(++) zone of inhibition between 1.1 - 1.7 cm
(+++) zone of inhibition > 1.7 cm

Antimicrobial activity of Protexin strains

Antimicrobial activity was detected for all the Protexin strains. All nine lactobacilli showed good inhibition of the four pathogens: *Salmonella typhimurium*, *Staphylococcus aureus*, *Escheria coli* and *Enterococcus faecalis*.

The probiotic *B. breve* can be seen to be an effective inhibitor of the pathogenic strains tested. The other bifidobacteria appeared to be less effective than the lactobacilli species, however it could be that these species possess other probiotic effects (e.g. strengthening the epithelial barrier).

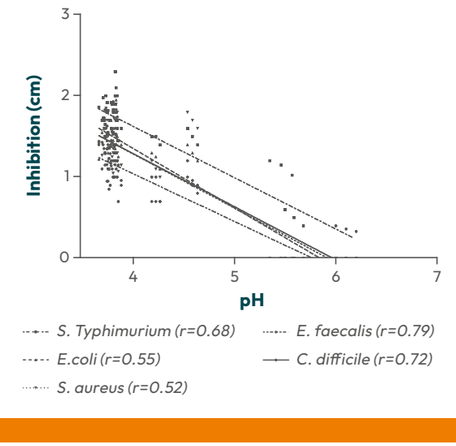
Inhibition effect of *Lactobacillus rhamnosus* PXN 54 on *Salmonella typhimurium*

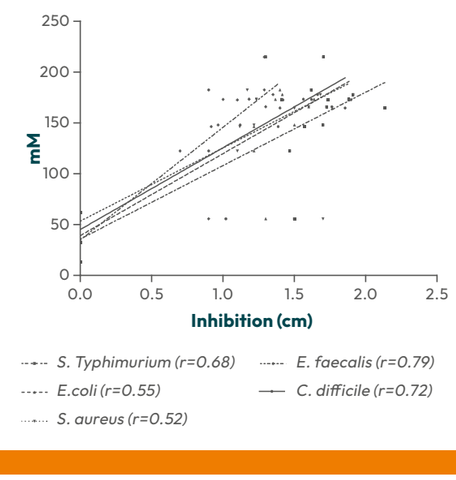


Well diffusion assays showed that there was more inhibition with non-adjusted pH than pH7 supernatant, suggesting that the most likely mechanism of action for the pathogen inhibition was acid production by the probiotic strains.

Acid production and antimicrobial activity

Most bacteria showed high production of lactic and acetic acid. The higher the concentration of lactic acid produced by bacteria, the greater the inhibition against each pathogen. In contrast, higher production of acetic acid did not did not significantly influence the inhibitory effect.





Haemolytic activity of Protexin strains

The data produced showed that none of the Protexin strains were found to be β-haemolytic. This is a positive result as haemolysis would not be a desired characteristic of probiotic strains as this is the splitting of red blood cells and the release of their contents into the blood plasma.

Probiotic hydrophobicity

Strain hydrophobicity is an indicator of adherence to epithelial cells – hydrophobicity is considered as one of the main physical interactions during bacterial adhesion to cell linings.

Results have shown that all of the Protexin strains are hydrophobic, with *L. casei* PXN 37, *L. acidophilus* PXN 35, *L. salivarius* PXN 57, *L. rhamnosus* PXN 54, *L. fermentum* PXN 44, *L. lactis* PXN 63, *B. bifidum* PXN 23, *B. infantis* PXN 27, *S. thermophilus* PXN 66 and *B. subtilis* PXN 21 found to be highly hydrophobic.

Conclusion

These findings show that there is significant antimicrobial activity of the strains tested and that a reduction in pH is responsible mainly for this activity (due to the production of acid from the probiotics). The results also show that the Protexin strains are not haemolytic and they have good cell adhesion properties.

Tejero-Sariñena S, Barlow J, Costabile A, Gibson GR, Rowland I. In vitro evaluation of the antimicrobial activity of a range of probiotics against pathogens: evidence for the effects of organic acids. *Anaerobe* 2012;**18**:530-8.

Antimicrobial activity of single and multi-strain probiotics against *C. difficile* and *S. typhimurium* – specificity or synergism

Objective

The aim of this *in vitro* study was to evaluate the influence of three single probiotics: *Lactobacillus casei* PXN 37, *Lactobacillus acidophilus* PXN 35, *Bifidobacterium breve* PXN 25 and a probiotic mixture containing the above strains plus twelve other strains belonging to the Lactobacillus, Bifidobacterium, Lactococcus, Streptococcus and Bacillus genera on the survival of *Salmonella typhimurium* and *Clostridium difficile*.

Methods

The strains were evaluated using pH-controlled anaerobic batch cultures containing mixed faecal bacteria. Changes in relevant bacterial groups and effects of probiotic addition on survival of the two pathogens were assessed over 24 hours.

Protexin strains demonstrated significant inhibition of *S. typhimurium* and *C.difficile*

Results

Quantitative analysis of bacterial populations revealed that there was a significant reduction in *S. typhimurium* and *C. difficile* numbers in the presence of probiotics compared with controls. Of the probiotic treatments, two single strains, namely *L. casei* PXN 37 and *B. breve* PXN 25, were the most potent in reducing the numbers of *S. typhimurium* and *C. difficile*. The probiotic mixture was equally as effective as the two most potent strains mentioned at reducing numbers of *S. typhimurium* and *C. difficile* despite having fewer numbers of those specific probiotics by volume.

Conclusion

These findings show that there is significant anti-microbial activity against *S. typhimurium* and *C. difficile* associated with Protexin strains, with PXN 25 & PXN 37 demonstrating particularly potent effects. These findings suggest that multi-strain mixtures can be as effective as those single strains alone. Taken in conjunction with other *in vitro* work this study can form part of the rationale for additional clinical trial work in infective and antibiotic associated diarrhoea (*C. difficile* is the most common pathogen associated with AAD).

Tejero-Sariñena S, Barlow J, Costabile A, Gibson GR, Rowland I. Antipathogenic activity of probiotics against *Salmonella typhimurium* and *Clostridium difficile* in anaerobic batch culture systems: Is it due to synergies in probiotic mixtures or the specificity of single strains? *Anaerobe* 2013;**24**:60-65.

Evaluation of *Bacillus subtilis* strains as probiotics

Objective

To evaluate spores of *B.subilis* including commercial Protexin strain PXN 21 for their potential value as a probiotic and as potential food additives. This included resistence to gastric fluid, sporulation efficiency, formation of biofilms and effect on the immune system.

Methods

Two isolates of *B.subtilis* examined were HU58 (human isolate) and PXN 21 against a laboratory strain – PY79. Sporulation was examined using the exhaustion method – inducing sporulation of all three strains and examining the spore counts. Biofilm formation was assessed looking at pellicle-like surface colonies on semi-solid media where different phases of colonisation could be assessed. Growth was assessed on MSgg medium, CM and CMK media.

Evaluation of the three spores through intestinal fluid was conducted in simulated gastric fluid (SGF) at pH 2, pH 3, and pH 4. Persistence of spores through the gastro-intestinal tract was measured following a single oral dose of each strain (1 x 10^9 CFU) to mice and examined faecal shedding.

Groups of inbred mice were also dosed orally (1 x 10^9 CFU) of PY79 or PXN 21 every 7 days for 10 weeks. At the end of the dosing regimen, splenocytes were cultured and stimulated with various exogenous antigens: *E.coli* LPS, CDTA of *C.difficile*, gluteraldehyde-inactivated *C.difficile* 630 spores, as well as two *C.difficile* spore coat proteins, CotC and CotD, and measurements of IFN-y were taken.

Results

Sporulation efficiency & mucin adhesion

PXN 21 showed the first detectable levels of heat resistant spores 2-3 hours post-induction and maximal counts at 8 hours. This closely resembled the human isolate HU58.

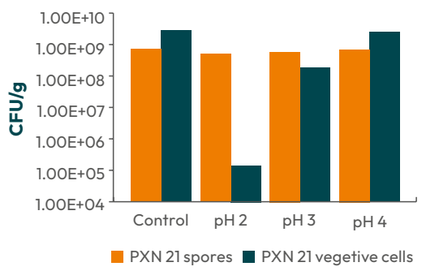
Biofilm formation

The ability of PXN 21 to adhere to mucin was also assessed and compared against previously characterised strains using the mucin adhesion assay. PXN 21 demonstrated a significantly greater adhesion efficacy than the reference strains by more than 2 logs.

Resistance to intestinal fluids

Spores exhibited almost no loss in viability after 1 hour of incubation. Vegetative cells were labile at pH 2 showing a 4-5 log reduction in CFU count after 1 hour incubation. However, at pH 4 (closely relating to the gastric pH after a full meal), vegetative cells showed no sensitivity to SGF and total viability was not affected.

Survival of PXN 21 spores and vegetative cells in simulated gastric fluid for 1 hour



Persistence of spores in the gastro-intestinal tract

Following a single dose of 1 x 10^9 CFU, PXN 21 spores were still found to be present 18 days post dosing in the faeces of mice. This further suggests that PXN 21 is better adapted to gut residency than some other tested strains and this correlates with the ability of the strain to produce biofilms. The robustness of PXN 21 through intestinal fluids suggests that both vegetative cells and spores should pass through the stomach unscathed if they are consumed with a meal.

Non-specific immune responses

PXN 21 immunised mice showed abundantly produced IFN-y when stimulated with a variety of antigens including *C.difficile* and *E.coli*. IFN-y is the primary cytokine involved in macrophage activation and mediating the host's defences to bacterial and viral pathogens. This result demonstrates the ability of PXN 21 spores to stimulate non-specific (innate) immunity.

Conclusion

PXN 21 is a fast sporulator and most closely resembles other natural isolates of *B.subtilis*. It is also able to adhere to mucin which is considered a beneficial feature to intestinal bacteria, enabling them to obtain nutrients and more efficiently colonise the mucosal epithelial layers. The ability of PXN 21 to create biofilms and persist in the GI tract demonstrates it's suitability to gut residency. This study also demonstrated the capability of PXN 21 to stimulate the immune system. All of this data supports the use of PXN 21 as a probiotic.

PXN 21 is able to colonise in the gut and stimulate non-specific (innate) immunity

Permpoonpattana P, Hong HA, Khaneja R, Cutting SM. Evaluation of Bacillus subtilis strains as probiotics and their potential as a food ingredient. *Benef Microbes* 2012;**3**:127-35.

The effect of *Bacillus subtilis* PXN 21 spores on ***C.difficile*** **infection**, murine model

PXN 21 spores improved survival rates in a *C .difficile* infection model

Objective

In this work, Protexin spores of *Bacillus subtilis* PXN 21, a bacterial species already widely associated with probiotic use, was used to suppress symptoms of *Clostridium difficile* infection (CDI). The objective was to assess the potential capacity of this bacterium, as a probiotic, to protect against disease using a murine model of infection.

Results

Colonisation resistance

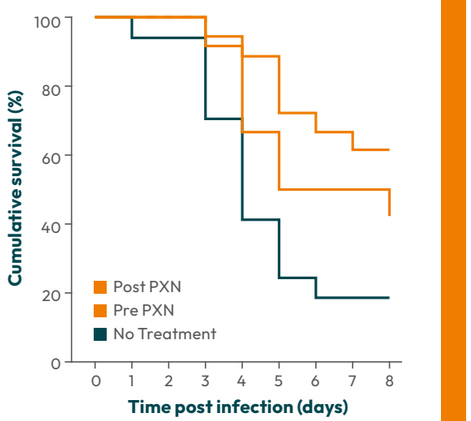
Levels of *C. difficile* spores present in faecal samples demonstrated that oral delivery of *B. subtilis* PXN 21 spores both prior to and post infection had no significant effect on reducing *C. difficile* colonisation.

Attenuation of symptoms in a model of fatal disease

Delivery of PXN 21 spores prior to infection resulted in a survival rate of 41.6% while 66.6% survival was achieved in animals treated post-CDI; this compared to a survival rate of 16.6% in non-treated groups. Weight profiles of infected animals added further complexity to the results. Animals that survived infection having received probiotic treatment prior to infection displayed less weight loss than those infected with PXN 21 spores post infection.

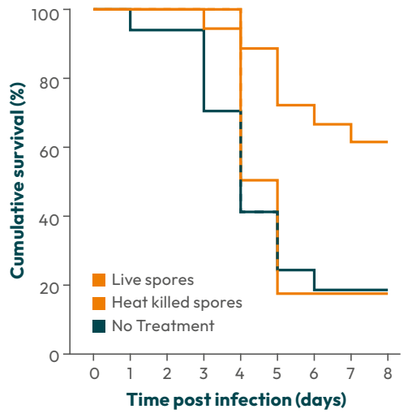
It was also shown that a dosing of mice post infection with killed spores showed almost no improvement on survival; by contrast, dosing with live spores markedly improved survival.

Delivery of PXN 21 spores pre and post CDI

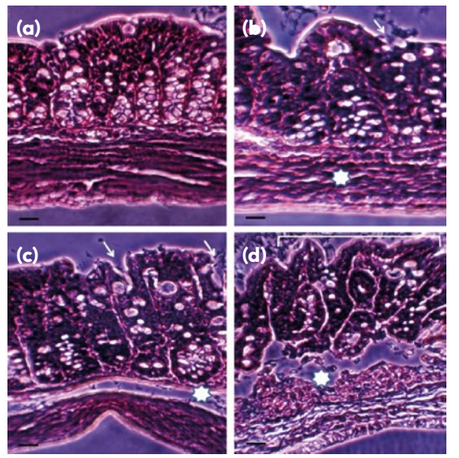


Pre PXN = Administration of *B. subtilis* PXN 21 prior to infection with *C. difficile*.
Post PXN = Administration of *B. subtilis* PXN 21 after infection with *C. difficile*.

Heat killed PXN 21 spores versus live PXN 21 spores against CDI



Histopathology



Haematoxylin and eosin (H&E) – stained sections of colon from *Clostridium difficile* VPI 10463 infected mice.

(a) Uninfected mice, displaying healthy tissue and intact epithelial lining to colon;

(b) mice treated with PXN 21 spores preinfection with some damage to epithelial structure but with lining still intact although evidence of mild oedema in the submucosa;

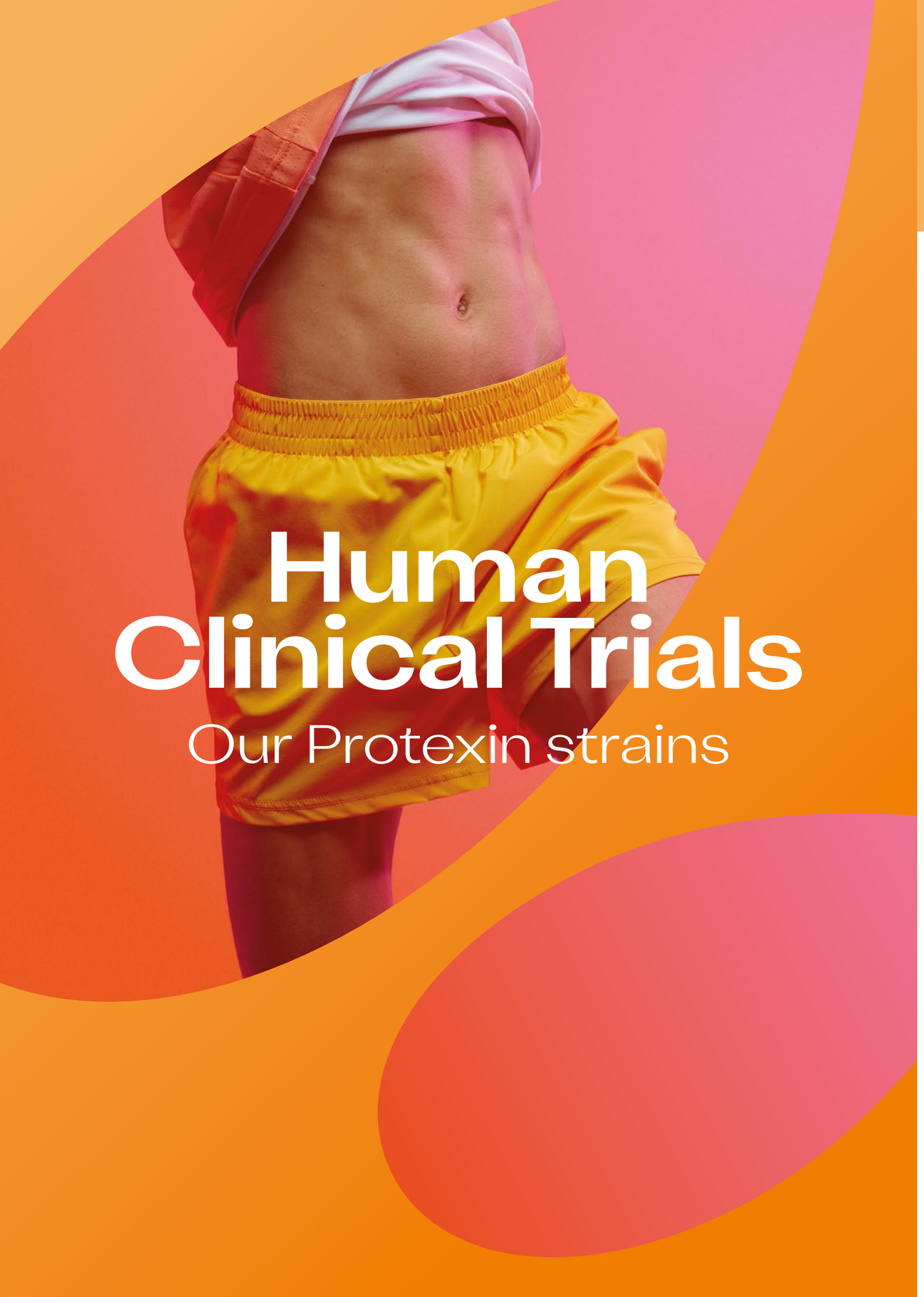
(c) mice treated with PXN 21 spores post infection showed some damage to the integrity of the epithelial lining and some submucosal disruption; and

(d) untreated mice displaying extensive damage to the colonic epithelial lining and erosion of the submucosa (shown by white arrows and stars).
Scale bars 25 µm.

Conclusion

This study demonstrates that live spores of *Bacillus subtilis* can attenuate the effects of *Clostridium difficile* infection in a murine model of disease by up to fourfold. The proposed mechanism of action is one through which the PXN 21 probiotic strain might stimulate the innate immune response. Suppression was not complete as animals were still infected but this work can form the basis for further studies to evaluate the effects of different doses of PXN 21 and ultimately the development of human clinical trials to assess the probiotic further.

Colenutt C, Cutting SM. Use of *Bacillus subtilis* PXN 21 spores for suppression of *Clostridium difficile* infection symptoms in a murine model. *FEMS Microbiol Lett* 2014;**358**:154-61.



Human Clinical Trials

Our Protexin strains

The effect of probiotics on **faecal calprotectin in patients with cystic fibrosis**, RCT

Objective

This study was performed to assess the effects of a Protexin probiotic formulation on intestinal inflammation in a group of children with cystic fibrosis (CF) as measured by faecal calprotectin levels.

The Protexin multi-strain formulation
Lactobacillus casei PXN 37
Lactobacillus rhamnosus PXN 54
Streptococcus thermophilus PXN 66
Bifidobacterium breve PXN 25
Lactobacillus acidophilus PXN 35
Bifidobacterium infantis PXN 27
Lactobacillus bulgaricus PXN 39
Fructooligosaccharide (FOS)
Total viable count per sachet 1 x 10^9 CFU
Daily dose: 1 sachet (1 x 10^9 CFU)

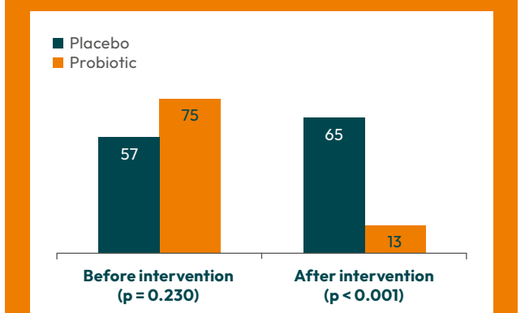
Methods

This was a double blind, randomised, placebo controlled trial in which 47 patients with cystic fibrosis were allocated to receive either a 7 strain probiotic formulation (24) or a placebo (23) for 4 weeks. Calprotectin levels were measured at baseline and after treatment to see if there was any effect. Patients were classified as having an abnormal or high calprotectin level if the value was above 50µg/g of faeces.

Results

31 of 47 enrolled patients (65.9%) were found to have abnormally high calprotectin levels; 13 in the placebo group and 18 in the probiotic group. This difference was not found to be statistically significant (p = 0.230) so both groups were comparable at baseline. Following the intervention period a significantly lower proportion of patients in the probiotic group were found to have high calprotectin levels than in the placebo group (p < 0.001). The proportion of patients with high calprotectin levels actually increased in the placebo group.

Percentage of patients with high calprotectin levels before and after treatment



Conclusion

This study showed that about two-thirds of patients with CF had intestinal inflammation based on faecal calprotectin levels. Administration of the Protexin probiotic formulation was shown to decrease calprotectin concentrations and intestinal inflammation in CF patients. This study supports the rationale for further clinical trials looking at the use of probiotics to help manage abdominal symptoms associated with cystic fibrosis.

Protexin probiotics significantly decreased intestinal inflammation in children with cystic fibrosis

Fallahi G, Motamed F, Yousefi A, *et al*. The effect of probiotics on fecal calprotectin in patients with cystic fibrosis. *Turk J Pediatr* 2013;**55**:475-8.

The clinical efficacy of probiotics in the management of **childhood atopic dermatitis**, RCT

Objective

To study the clinical and immunologic effects of a Protexin probiotic formulation in infants and children with atopic dermatitis (AD).

The Protexin multi-strain formulation
Lactobacillus casei PXN 37
Lactobacillus rhamnosus PXN 54
Streptococcus thermophilus PXN 66
Bifidobacterium breve PXN 25
Lactobacillus acidophilus PXN 35
Bifidobacterium infantis PXN 27
Lactobacillus bulgaricus PXN 39
Fructooligosaccharide (FOS)
Total viable count per sachet 1 x 10^9 CFU
Daily dose: 1 sachet (1 x 10^9 CFU)

Methods

A randomised, double blind, placebo controlled study with 40 infants and children aged 3 months to 6 years with mild to severe AD. The infants and children received either the Protexin multi-strain probiotic or placebo for eight weeks. The Severity Scoring of Atopic Dermatitis (SCORAD) index was recorded at baseline and also at four and eight weeks. Allergic sensitisation was evaluated by measurement of total IgE at two points and skin prick test for common food allergens at baseline. IL-4 and IFN-gamma concentrations were measured from pre and post treatment blood samples.

Results

Both groups received optimal skin care treatment for AD, but the Protexin group showed a significantly greater reduction in SCORAD than the placebo group within first 4 weeks (30% vs 10%) and 8 weeks (40% vs 20%). However, the mean difference in IgE between the two groups was not statistically significant and no changes in cytokine profiles were detected.

Conclusion

This study provides evidence that the Protexin probiotic formulation clinically improves the severity of AD in young children. In 8 weeks, use of Protexin probiotics helped to reduce severity of atopic dermatitis by 40%.

Protexin probiotics significantly reduced symptoms of eczema in children

Farid R, Ahanchian H, Jabbari F, Moghiman T. Effect of a new synbiotic mixture on atopic dermatitis in children: a randomized-controlled trial. *Iran J Pediatr* 2011;**21**:225-30.

The clinical efficacy of probiotics in **paediatric acute gastroenteritis**, RCT

Protexin probiotics significantly reduced duration of infectious diarrhoea in children

Objective

To determine the clinical efficacy of Protexin probiotics in reducing duration of acute diarrhoea in children with acute gastroenteritis (AGE).

The Protexin multi-strain formulation
Lactobacillus casei PXN 37
Lactobacillus rhamnosus PXN 54
Streptococcus thermophilus PXN 66
Bifidobacterium breve PXN 25
Lactobacillus acidophilus PXN 35
Bifidobacterium infantis PXN 27
Lactobacillus bulgaricus PXN 39
Fructooligosaccharide (FOS)
Total viable count per sachet 1 x 10^9 CFU
Daily dose: 1 sachet (1 x 10^9 CFU)

Methods

In this randomised, open label, controlled trial of 101 paediatric patients aged 6 months to 12 years, with suspected viral acute gastroenteritis (AGE) were enrolled. Almost 50% of patients were aged 6 months to 2 years. Standard supportive therapy which included oral rehydration and/or diet was initiated in all patients, with the synbiotic group also receiving the Protexin multi-strain probiotic daily for 7 days. A daily diary was used to record the number and consistency of stools (Bristol Stool Chart), as well as other symptoms like fever, vomiting, abdominal pain, adverse events and time of first normal stool. The parents were contacted by phone on days 2, 4 and 7 to monitor changes in symptoms, and on day 14 (7 days after the last probiotic dose) to assess adverse events.

Results

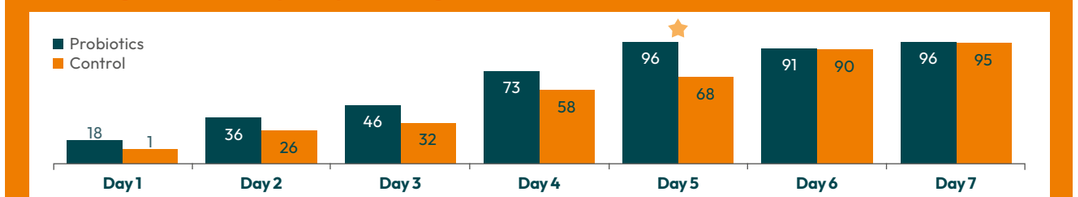
In total, 43 children in the synbiotic group and 42 in the control group completed the study. Almost 50% of patients were aged 6 months to 2 years. The proportion of patients without diarrhoea over the study period was greater in the synbiotic group at all time points. Overall, duration of diarrhoea was reduced by 1 day in the synbiotic group. In children aged up to 2 years the effect was even greater, with the duration shortened by 2 days in the synbiotic group (p=0.034).

Number of additional visits to the doctors after study enrolment in the synbiotic group was almost half that of the control group. The frequency of adverse events was similar between the groups. Compliance with the synbiotic was 90%.

Conclusions

The supplementation of Protexin multi-strain formulation to children with AGE reduced duration of diarrhoea by an average of 1 day, and by two days in infants up to 2 years of age. Administration of the probiotic formulation with standard hydration therapy was well perceived by parents.

Percentage of children (up to 2 years of age) without diarrhoea (%)



García-Menor E, García-Marín F, Vecino-López R, *et al.* A Multicenter, Prospective, Randomized Controlled Trial to Evaluate the Additional Benefit of a Multistrain Synbiotic (Prodefen®) in the Clinical Management of Acute Viral Diarrhea in Children. *Glob Pediatr Heal* 2016;**3**:2333794X16679587.

The clinical efficacy of probiotics in the management of **acute gastroenteritis in children**

Objective

To determine the clinical efficacy of a Protexin probiotic formulation as adjunct treatment of acute gastroenteritis.

The Protexin multi-strain formulation
Lactobacillus casei PXN 37
Lactobacillus rhamnosus PXN 54
Streptococcus thermophilus PXN 66
Bifidobacterium breve PXN 25
Lactobacillus acidophilus PXN 35
Bifidobacterium infantis PXN 27
Lactobacillus bulgaricus PXN 39
Fructooligosaccharide (FOS)
Total viable count per sachet 1 x 10^9 CFU
Daily dose: 1 sachet (1 x 10^9 CFU)

Methods

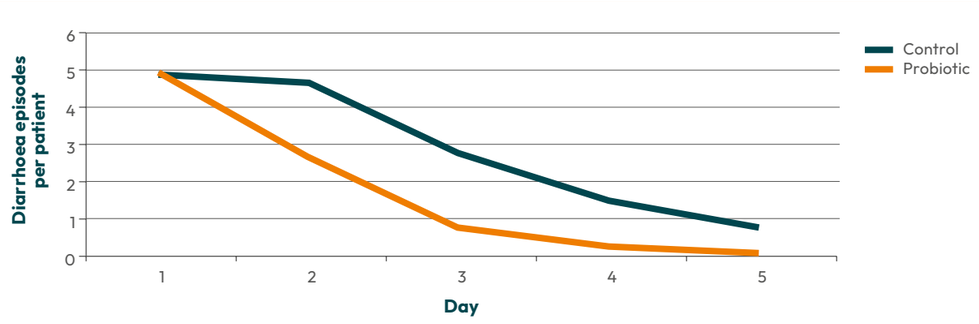
51 children aged from 2 months to 2 years were included in this randomised, single blind clinical trial where they were assigned to either standard treatment group (control group) or standard treatment plus probiotics (probiotics group). Frequency of diarrhoea, stool grade, length of hospital stay and any adverse reactions were reported.

Standard treatment was used according to WHO guidelines; the use of oral rehydration solution, intravenous fluid if indicated, and zinc supplementation.

Results

The probiotic group showed a decline in diarrhoea rate which was significant on the third day. Improvement in stool consistency was significant in the probiotic group on the second hospital day and the probiotic group had a significantly shorter course of hospitilisation of at least one day when compared to the control group. No adverse reactions were reported from either group.

Mean frequency of diarrhoea in both study groups



Conclusion

The Protexin probiotic formulation is both efficacious and safe in patients 2 months to 2 years old with acute gastroenteritis and should be considered as an additional therapeutic modality in the treatment of gastroenteritis.

Protexin probiotics significantly improved recovery in infants suffering with acute diarrhoea

Yala ET. The clinical efficacy of multi-strain probiotics (Protexin) in the management of acute gastroenteritis in children two months to two years old. *Pidsp* 2010;**11**:86–91.

The clinical efficacy of probiotics in the management of **infantile colic**, RCT

Objective

To study the effects of a Protexin probiotic formulation in the management of infantile colic.

The Protexin multi-strain formulation
Lactobacillus casei PXN 37
Lactobacillus rhamnosus PXN 54
Streptococcus thermophilus PXN 66
Lactobacillus acidophilus PXN 35
Bifidobacterium breve PXN 25
Lactobacillus bulgaricus PXN 39
Bifidobacterium infantis PXN 27
Fructooligosaccharide (FOS)
Total viable count per sachet 1 x 10^9 CFU
Daily dose: 1 sachet (1 x 10^9 CFU)

Methods

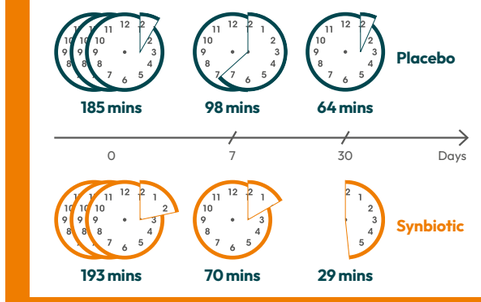
This was a randomised, double blind, placebo controlled, prospective, parallel arm study in 50 infants aged 2 weeks to 4 months diagnosed with infantile colic as defined by Wessel's criteria. The infants were randomly assigned to receive placebo or the probiotic mixture for 30 days. The primary objective was treatment success, defined as a reduction in average daily crying of at least 50% and the secondary objective was resolution of symptoms, defined as an average 90% reduction in daily crying. The primary and secondary endpoints were measured at 7 days after starting active treatment and at 30 days using symptom diaries completed by the parents.

Results

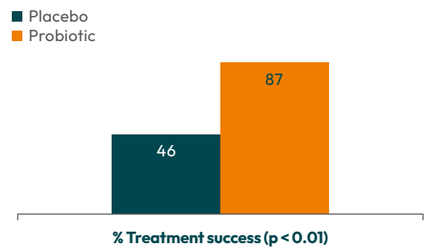
45 infants completed the study. The two groups were comparable, with no difference seen in baseline characteristics. Treatment success was significantly higher in the probiotic group (82.6%) compared with the placebo group (35.7%), at day 7 ($p < 0.005$). This difference was maintained at day 30 with a treatment success of 87% and 46% in the probiotic group and placebo group, respectively ($p < 0.01$). In addition, symptom resolution was significantly higher in the probiotic group (39%) compared to the placebo group (7%) at day 7 ($p < 0.03$). This significant difference was not maintained at day 30 (56% vs 36%, $p = 0.24$). No adverse events were reported.

At the end of the study period infants in probiotic group cried on average 30 minutes less per day than infants in the placebo group.

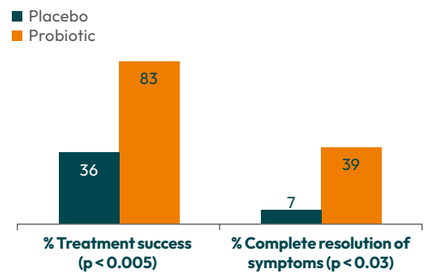
Cry & fuss time



Outcomes after 30 days of treatment



Outcomes after 7 days of treatment



Conclusion

This study provides evidence that the Protexin probiotic formulation used can help to manage symptoms of infantile colic without any reported side effects. Crying time was reduced by half in 8 out of 10 infants in the probiotic group within the first 7 days.

Protexin probiotics significantly reduced infant colic

Kianifar H, Ahanchian H, Grover Z, *et al.* Synbiotic in the management of infantile colic: a randomised controlled trial. *J Paediatr Child Health* 2014;**50**:801-5.

The clinical efficacy of probiotics in the treatment of **paediatric *H. pylori* infection**, RCT

Protexin probiotics significantly increased *H. pylori* eradication rates in children

Objective

The aim of this study was to determine whether adding a Protexin probiotic formulation to standard *Helicobacter pylori* eradication therapy could minimize the gastrointestinal side-effect prevalence and improve the eradication rate.

The Protexin multi-strain formulation
Lactobacillus casei PXN 37
Lactobacillus rhamnosus PXN 54
Streptococcus thermophilus PXN 66
Lactobacillus acidophilus PXN 35
Bifidobacterium breve PXN 25
Lactobacillus bulgaricus PXN 39
Bifidobacterium infantis PXN 27
Fructooligosaccharide (FOS)
Total viable count per sachet 1 x 10^9 CFU
Daily dose: 1 sachet (1 x 10^9 CFU)

Methods

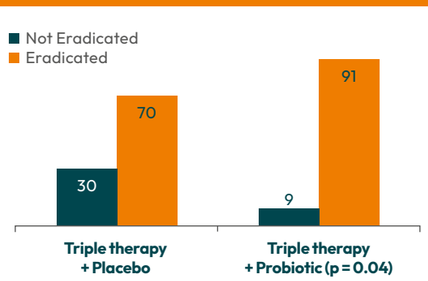
This was a double-blind, randomised, placebo controlled study conducted in 66 *H. pylori* positive children age 3 to 14 years. They were all treated with the standard triple drug therapy protocol (omeprazole+amoxycillin+flurazolidone) and randomly allocated to receive either probiotic or placebo. All patients underwent gastroscopy. *H. pylori* infection was diagnosed by either rapid urease test (RUT) or histology. *H. pylori* status was assessed after completion of treatment and eradication rate was assessed. Side effects and adverse events were also recorded.

Results

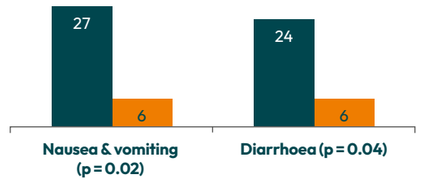
Both groups were comparable with no differences in baseline characteristics. All 66 patients completed the course of treatment and follow-up. The rate of *H. pylori* eradication was significantly higher in the probiotic group (P=0.04). In the probiotic supplemented children there was a lower rate of nausea & vomiting (P=0.02) and diarrhoea (P=0.04) during treatment. No other serious adverse events were noted.

Protexin probiotics significantly reduced diarrhoea associated with antibiotics in children

Eradication of *H. pylori* (%)



Side effect rates (%)



Conclusion

This double blind, randomised, placebo controlled study demonstrates that the Protexin probiotic formulation used is potentially an excellent adjuvant therapy to help improve eradication of *H. pylori* alongside conventional optimal treatment.

Adjuvant treatment with Protexin probiotics in triple therapy for *H. pylori* reduced side effect by 75% and increased eradication rate by 20%.

Khodadad A, Farahmand F, Najafi M, Shoaran M. Probiotics for the treatment of pediatric *Helicobacter pylori* infection: a randomized double blind clinical trial. *Iran J Pediatr* 2013;**23**:79–84.

The clinical efficacy of probiotics as adjunct treatment in **neonatal pneumonia**, RCT

Protexin probiotics significantly reduced sepsis and hospital stay in babies with neonatal pneumonia

Objective

To determine the effect of a Protexin probiotic formulation as an adjunct in the treatment of neonatal pneumonia.

The Protexin multi-strain formulation
Lactobacillus casei PXN 37
Lactobacillus rhamnosus PXN 54
Streptococcus thermophilus PXN 66
Bifidobacterium breve PXN 25
Lactobacillus acidophilus PXN 35
Bifidobacterium infantis PXN 27
Lactobacillus bulgaricus PXN 39
Fructooligosaccharide (FOS)
Total viable count per sachet 1 x 10^9 CFU
Daily dose: 1 sachet (1 x 10^9 CFU)

Methods

Thirty newborn babies were included in this randomised, controlled study. The inclusion criteria for the study was newborns 0 – 28 days old who were admitted to the neonate intensive care unit (NICU) diagnosed with pneumonia.

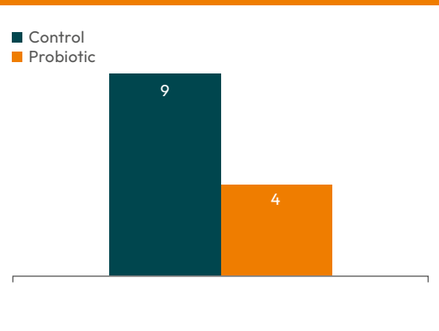
The babies were then randomised to conventional treatment and IV antibiotics (control group) or conventional treatment, IV antibiotics plus one sachet of Protexin multi-strain probiotics per day (probiotic group).

Symptoms of respiratory distress were measured such as rapid breathing and labored breathing as well as the length of hospital stay.

Results

The results of this study showed a statistically significant difference in rapid breathing, with subjects in group B (probiotic group) having a shorter duration of rapid breathing ($p < 0.001$). Differences were also seen with laboured breathing, early feeding tolerance (probably secondary to the shortened duration of respiratory distress) and length of hospital stay, with the probiotic group showing significant reductions when compared to the control group. This also led to a significant reduction in neonatal sepsis in the experimental group (6.6%), compared to 66% in the control group.

Average length of hospital stay in both groups (days)



Conclusion

The supplementation of the Protexin probiotic formulation with IV antibiotics in patients admitted with neonatal pneumonia showed a significant difference in reducing the duration of symptoms of respiratory distress like rapid breathing and laboured breathing. Early feeding in neonatal pneumonia is one of the problems that is usually encountered in patients admitted either in the NICU or pediatric ward. This study showed that using the Protexin probiotic formulation as an adjunct to the treatment of neonatal pneumonia significantly reduces the length of symptoms associated with the condition and halves hospital stay.

ADM Protexin: *Data on file 4.*

The clinical efficacy of probiotics in **childhood constipation**, RCT 1

Objective

To evaluate the effectiveness of a Protexin probiotic formulation on childhood chronic functional constipation.

The Protexin multi-strain formulation
Lactobacillus casei PXN 37
Lactobacillus rhamnosus PXN 54
Streptococcus thermophilus PXN 66
Bifidobacterium breve PXN 25
Lactobacillus acidophilus PXN 35
Bifidobacterium infantis PXN 27
Lactobacillus bulgaricus PXN 39
Fructooligosaccharide (FOS)
Total viable count per sachet 1 x 10⁹ CFU
Daily dose: 1 sachet (1 x 10⁹ CFU)

Methods

102 children with functional constipation diagnosed with Rome III criteria aged between 4 and 12 years participated in this double-blind, randomised, placebo controlled trial. The children were allocated into 3 groups:

A) 1.5ml/kg/day oral liquid paraffin plus powder placebo per day
B) 1 sachet of Protexin probiotic per day plus liquid placebo
C) 1.5ml/kg/day oral liquid paraffin plus 1 sachet of Protexin probiotic per day

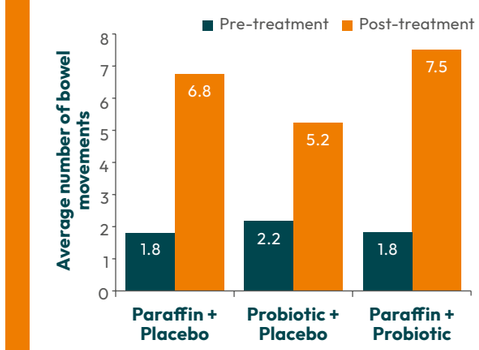
The groups were studied for 4 weeks with bowel movements (BMs), stool consistency, faecal incontinence, abdominal pain and painful defecation as the primary outcomes. The secondary outcomes were incidence of adverse effects and success of treatment, which was determined as more than three BMs per week and less than two faecal incontinence episodes per month and no abdominal pain. No other medications were used during the study.

Results

97 children completed the study. The results showed that there was a significant increase in the number of BMs per week in all study groups, but the highest rise ($p = 0.03$) was in group C (liquid paraffin & probiotic group). Improvement in stool consistency and decrease in number of faecal incontinence episodes happened in all three groups without any statistical significance between all three.

There were no side effects reported in group B (probiotic only group) and this was significantly different to the other two groups which reported 18 side effects in group A and 21 in group C.

Frequency of bowel movements per week before and after treatment



Conclusion

This double-blind randomised placebo controlled trial showed that the Protexin probiotic formulation has positive effects on symptoms of childhood constipation without any side effects.

Khodadad A, Sabbaghian M. Role of synbiotics in the treatment of childhood constipation: a double-blind randomized placebo controlled trial. *Iran J Pediatr* 2010;**20**:387-392.

The clinical efficacy of probiotics in **childhood constipation**, RCT 2

Objective

To study the effects of a Protexin probiotic formulation on symptoms associated with chronic constipation in children.

The Protexin multi-strain formulation
Lactobacillus casei PXN 37
Lactobacillus rhamnosus PXN 54
Streptococcus thermophilus PXN 66
Lactobacillus acidophilus PXN 35
Bifidobacterium breve PXN 25
Lactobacillus bulgaricus PXN 39
Bifidobacterium infantis PXN 27
Fructooligosaccharide (FOS)
Total viable count per sachet 1 x 10⁹ CFU
Daily dose: 1 sachet (1 x 10⁹ CFU)

Protexin probiotics significantly reduced childhood constipation

Methods

This was a randomised, double blind, placebo controlled prospective parallel arm study in 56 children aged between 4 – 12 years diagnosed with chronic constipation as per Rome III criteria. Patients were randomly assigned to receive lactulose + probiotic or lactulose + placebo for a total of 4 weeks. The study assessed changes in stool frequency, consistency, abdominal pain and adverse events.

Results

The two groups were comparable with no differences seen in baseline characteristics. After the intervention period, improvements were seen in both consistency and frequency of stool in both groups. However, the probiotic group had a significantly ($p = 0.042$) greater increase in frequency of stool (2.08 bowel motions per week) compared to the placebo group (1.54 bowel motions per week). There was also a significantly better improvement in stool consistency within the probiotic group compared to placebo ($p = 0.049$). Improvements were also seen in abdominal pain ($p = 0.017$) and faecal incontinence ($p = 0.03$) in the group taking the probiotic formulation. Improvement in faecal incontinence was significant after first 7 days in the probiotic, but not in the placebo group. No side effects or adverse events were noted.

Conclusion

This study provides evidence that the Protexin probiotic formulation can help to improve stool frequency, stool consistency, abdominal pain and faecal incontinence in children with chronic constipation, with some of the results seen within the first 7 days.

Sadeghzadeh M, Rabieefar A, Khoshnevisasl P, Mousavinasab N, Eftekhari K. The effect of probiotics on childhood constipation: a randomized controlled double blind clinical trial. *Int J Pediatr* 2014;**2014**:937212.

The clinical efficacy of probiotics in prevention of **necrotising enterocolitis in preterm infants**, RCT

Protexin probiotics significantly reduced incidence of necrotising enterocolitis in preterm infants

Objective

To determine the clinical efficacy of Protexin probiotics in prevention of necrotising enterocolitis (NEC) in preterm and very low weight infants.

The Protexin multi-strain formulation
Lactobacillus casei PXN 37
Lactobacillus rhamnosus PXN 54
Streptococcus thermophilus PXN 66
Bifidobacterium breve PXN 25
Lactobacillus acidophilus PXN 35
Bifidobacterium infantis PXN 27
Lactobacillus bulgaricus PXN 39
Fructooligosaccharide (FOS)
Total viable count per sachet 1 x 10^9 CFU
Daily dose: 1 sachet (1 x 10^9 CFU)

Methods

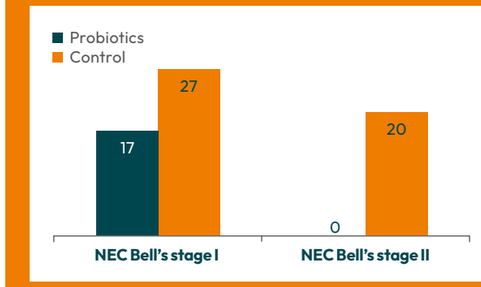
In this randomised, double blind, controlled trial 60 premature new-borns weighing 750-1500g or <32 weeks of gestation age were divided into two groups: probiotic and control. The probiotic group received 1 g of multi-strain probiotic given with enteral feeding in 8 to 10 divided doses for minimum 7 days. The control group received enteral nutrition without probiotic. Occurrence of NEC was monitored and categorised according to Bell's stages (I -suspected, II -definite, and III -advanced). Weight gain, days of total parenteral nutrition, days of full feeding achievement, days of hospitalisation, and rise in CRP were also monitored.

Results

Baseline characteristics including gestational age, 5th-minute Apgar score and need for resuscitation were similar in both groups. Incidence of NEC was significantly higher in the control group (p=0.02). Four out of five infants in the probiotic group who were diagnosed with suspected NEC (Bell's stage I) improved with continued probiotic feeding.

Rise in CRP, suggesting systemic inflammation, was seen in over 4 times more infants in the control group than in the probiotic group (30% vs 7%). The duration of oxygen therapy, days of TPN, days of full feeding achievement and duration of hospitalisation were not different between the two groups. Use of the probiotic formulation in divided doses with enteral feeds was practical.

Incidence of NEC in both groups (%)



Conclusions

The supplementation of the Protexin multi-strain formulation to preterm and very low birth weight infants, compared to placebo, showed a decreased incidence of NEC stage I and prevention of NEC stage II. Administration of the probiotic formulation with enteral feeds in divided doses was demonstrated to be successful.

Amini E, Dalili H, Niknafs N, Shariat M, Nakhostin M, Jedari-Attari S. The Effect of Probiotics in Prevention of Necrotising Enterocolitis in Preterm Neonates in Comparison with Control Group. *Iranian Journal of Pediatrics.* 2017;**27**(6).

The clinical efficacy of probiotics on **breast milk mineral content and infant growth**, RCT

Use of Protexin probiotics prevented reduction of breast milk mineral content

Objective

To evaluate the effect of a Protexin probiotics formulation on breast milk mineral composition and infant growth.

The Protexin multi-strain formulation
Lactobacillus acidophilus PXN 35
Lactobacillus casei PXN 37
Lactobacillus bulgaricus PXN 39
Lactobacillus rhamnosus PXN 54
Streptococcus thermophilus PXN 66
Bifidobacterium breve PXN 25
Bifidobacterium longum PXN 30
Fructooligosaccharide (FOS)
Total viable count per capsule 2 x 10^8 CFU
Daily dose: 2 capsules (4 x 10^8 CFU)

Methods

In this randomised, double blind, placebo controlled trial 70 lactating women who exclusively breast-fed their infants aged 90-120 days (full term birth and normal birth weight), were enrolled. Mothers received either probiotic or placebo capsules for 30 days. All measurements were performed at baseline and at the end of the intervention. Food intake was assessed with 24h recall for 2 days and food questionnaire for 1 day before the supplementation and at the end of the study. Breast milk samples were collected and analysed for mineral content by flame atomic absorption spectrometry.

Results

57 mothers completed the study. Baseline characteristics were not significantly different between the groups with the exception of length (shorter infants in the synbiotic group). During the study period the mean breast milk levels of zinc, copper, iron, magnesium and calcium insignificantly increased in the probiotic group, while the mean levels of minerals in the placebo group decreased significantly. After adjustment for maternal age, BMI and food intake, no significant associations were found between mineral intake and breast milk mineral content.

Duration of breast milk intake showed minimal increase of breast feeding time in the probiotic group (+2 min/24h) and a significant reduction in the placebo group (-24min/24h; p=0.01).

The comparison of changes in breast milk mineral content between the study groups after the intervention (mg/L)

	Synbiotic	Placebo
Zinc	+0.11	-0.60
Copper	+0.05	-0.16
Iron	+0.03	-0.18
Magnesium	+0.03	-0.17
Calcium	+0.90	-20.10

Conclusions

The supplementation of the Protexin multi-strain probiotic formulation to breastfeeding mothers prevented the reduction of mineral levels in breast milk and increased the duration of breast feeding. Since in this study no correlation was observed between mineral intake and breast milk mineral levels, it can be assumed that these positive changes could be due to synbiotic supplementation.

Mahdavi R, Taghipour S, Ostadrahimi A, Nikniaz L, Hezaveh SJG. A pilot study of synbiotic supplementation on breast milk mineral concentrations and growth of exclusively breast fed infants. *J Trace Elem Med Biol* 2015;**30**:25-29

The clinical efficacy of probiotic as an adjunct treatment in **metabolic syndrome**, RCT

Objective

The aim of this study was to evaluate the effects of a Protexin probiotic formulation alongside conventional lifestyle recommendations on insulin resistance and lipid profiles in individuals with the metabolic syndrome.

Methods

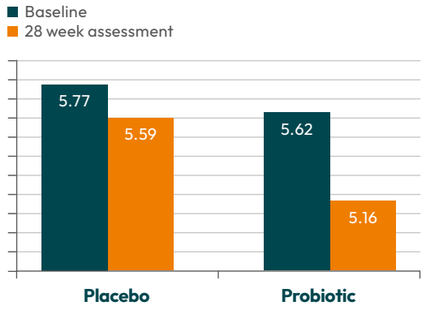
The Protexin multi-strain formulation
Lactobacillus casei PXN 37
Lactobacillus rhamnosus PXN 54
Streptococcus thermophilus PXN 66
Bifidobacterium breve PXN 25
Lactobacillus acidophilus PXN 35
Bifidobacterium longum PXN 30
Lactobacillus bulgaricus PXN 39
Fructooligosaccharide (FOS)
Total viable count per capsule 1 x 10^8 CFU
Daily dose: 2 capsules per day (2 x 10^8 CFU)

The study was a prospective, randomised, double-blind, placebo-controlled clinical trial. Men and women aged 18 years and above with a diagnosis of metabolic syndrome were randomised to receive either the probiotic preparation or placebo for 7 weeks alongside counselling to follow an energy-balanced diet and physical activity recommendations based on standardised clinical guidelines for the management of metabolic syndrome. Multiple anthropometric and biochemical parameters were assessed at baseline and at 7 weekly intervals for a total of 28 weeks.

Results

38 patients were enrolled into the study and randomised into two groups of 19. Baseline characteristics were comparable with no difference seen in any of the measured parameters. Both groups showed improvements in said parameters but the probiotic group showed greater improvements and this was maintained through to the 28 week assessment suggesting that the probiotic formula had long lasting beneficial effects.

Changes in fasting blood sugar (p<0.001) (mmol/l)



At baseline the difference between the two groups was not significant (p=0.731) but, whilst both groups demonstrated improvements, there was a significant difference between the two (p<0.001) after treatment.

Statistically significant differences were also seen after 28 weeks in triglyceride (p<0.001), HDL (p<0.001) and total cholesterol levels (p=0.010). Significant improvements were also seen in the insulin resistance (HOMA-IR) index and quantitative insulin sensitivity check index (QUICKI) during and after treatment.

Conclusion

This randomised, double-blind, placebo controlled study has found some evidence that probiotic supplementation augments the effects of lifestyle modification in the treatment of metabolic syndrome at least partially through the attenuation of insulin resistance and serum lipid levels. Further, larger and long term studies to evaluate clinical outcomes and quality of life measures are warranted to assess the long term use of probiotics as an adjunct to treatment in metabolic syndrome.

Protexin probiotics significantly reduced fasting blood sugar levels in patients being treated for metabolic syndrome

Eslamparast T, Zamani F, Hekmatdoost A, *et al.* Effects of synbiotic supplementation on insulin resistance in subjects with the metabolic syndrome: a randomised, double-blind, placebo-controlled pilot study. *Br J Nutr* 2014;**112**:438-45.

The clinical efficacy of probiotics as an adjunct treatment in **non-alcoholic fatty liver disease**, RCT

Protexin probiotics significantly improved liver function in patients with non-alcoholic fatty liver disease

Objective

Non-alcoholic fatty liver disease (NAFLD) is the most common chronic liver disease in the world. Oral administration of synbiotics has been proposed as an effective treatment of NAFLD because of its modulating effect on the gut flora, which can influence the gut-liver axis. This study was designed to evaluate the effects of supplementation with a a Protexin probiotic formulation on hepatic fibrosis, liver enzymes, and inflammatory markers in patients with NAFLD.

The Protexin multi-strain formulation
Lactobacillus casei PXN 37
Lactobacillus rhamnosus PXN 54
Streptococcus thermophilus PXN 66
Bifidobacterium breve PXN 25
Lactobacillus acidophilus PXN 35
Bifidobacterium longum PXN 30
Lactobacillus bulgaricus PXN 39
Fructooligosaccharide (FOS)
Total viable count per capsule 2 x 10^8 CFU
Daily dose: 1 capsule (2 x 10^8 CFU)

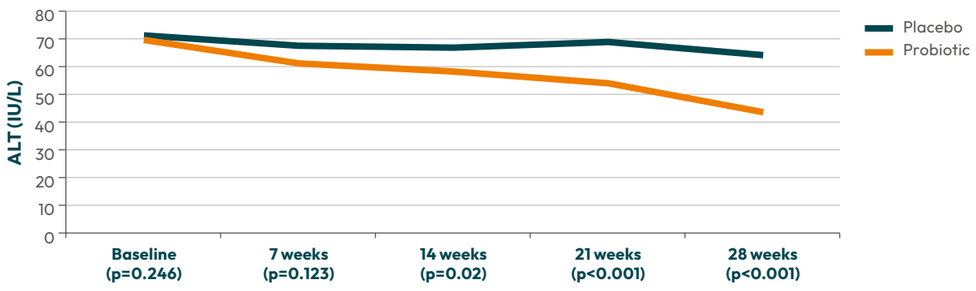
Methods

This was a randomised, double-blind, placebo-controlled clinical trial conducted as a pilot study in adult patients with NAFLD who were supplemented twice daily for 28 weeks with either the probiotic or a placebo capsule. Both groups were advised to follow an energy-balanced diet and physical activity recommendations according to standardised clinical guidelines. Multiple anthropometric and biochemical parameters were assessed at baseline and at seven-weekly intervals for a total of 28 weeks. The primary outcome was determined as improvement in hepatic function based on reduction of hepatic enzymes.

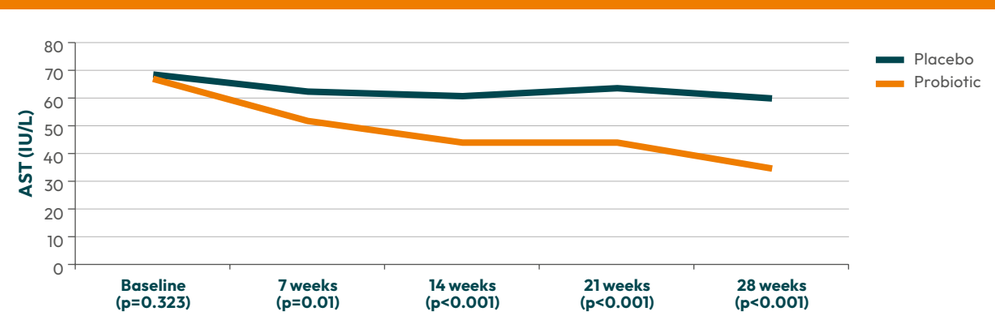
Results

Fifty two patients were enrolled into this study and randomised into the two groups. Baseline characteristics were comparable with no significant differences found between the two groups. Both groups showed improvements in alanine transaminase (ALT) as would be expected given the lifestyle changes implemented ($p<0.001$). However, the mean reduction in the probiotic group was significantly greater than that in the placebo group ($P<0.001$). Similar results were noted in the levels of aspartate transaminase (AST). Significant changes were observed at 21 and 14 weeks respectively, and maintained right through the 28 week assessment period.

Cummulative changes in alanine transaminase (ALT) levels over time (IU/L)



Cummulative changes in aspartate transaminase (AST) levels over time (IU/L)



Statistically significant improvements were also seen in both groups in Gamma-glutamyl transpeptidase (GGT) levels after 28 weeks with the mean improvement statistically greater in the probiotic group ($p<0.001$). A similar result was obtained when looking at improvements in the inflammatory markers hs-CRP and TNF-α with the mean improvement in both parameters greater in the probiotic group ($p<0.001$). Whilst there were also improvements in both BMI and waist to hip ratio in both groups the difference between the two groups was not significant ($p=0.13$).

Conclusion

This randomised, double-blind, placebo-controlled trial found some evidence that probiotic supplementation in addition to lifestyle modification is superior to lifestyle modification alone for the treatment of NAFLD, at least partially through attenuation of inflammatory markers in the body. Whether these effects will be sustained with longer treatment durations remains to be determined but this trial can form the basis for further, larger and longer term studies to conclusively evaluate the potential for the use of probiotics in the management of NAFLD.

Eslamparast T, Poustchi H, Zamani F, Sharafkhah M, Malekzadeh R, Hekmatdoost A. Synbiotic supplementation in nonalcoholic fatty liver disease: a randomized, double-blind, placebo-controlled pilot study. *Am J Clin Nutr* 2014;**99**:535-42.

The clinical efficacy of probiotics in the treatment of **non-alcoholic steatohepatitis**, RCT

Protexin probiotics significantly improved liver function in patients with non-alcoholic steatohepatitis

Objective

To assess the effects of a Protexin probiotic formulation alongside conventional treatment for non-alcoholic steatohepatitis (NASH). The study primarily investigated the effects on liver enzymes & ultrasound grading of steatosis.

The Protexin multi-strain formulation
Lactobacillus casei PXN 37
Lactobacillus rhamnosus PXN 54
Streptococcus thermophilus PXN 66
Bifidobacterium breve PXN 25
Lactobacillus acidophilus PXN 35
Bifidobacterium longum PXN 30
Lactobacillus bulgaricus PXN 39
Fructooligosaccharide (FOS)
Total viable count per capsule 1 x 10^8 CFU
Daily dose: 2 capsules (2 x 10^8 CFU)

Methods

This was a double blind, randomised, placebo controlled study in 67 patients aged between 18 – 75 years with histologically confirmed NASH. Patients were randomised to take either Metformin + Probiotics or Metformin + placebo for a total of 6 months. This was alongside normal conventional recommendations for management of NASH (weight control, exercise, diet changes). Liver function was assessed using measurements of hepatic enzymes and ultrasound was performed to grade the extent of the liver disease. This was done at baseline and after treatment with monitoring throughout the 6 month period.

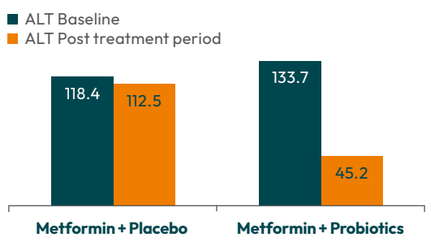
Results

Both groups were comparable with no difference in baseline characteristics. After the treatment period there were improvements in all parameters in both groups as would be expected.

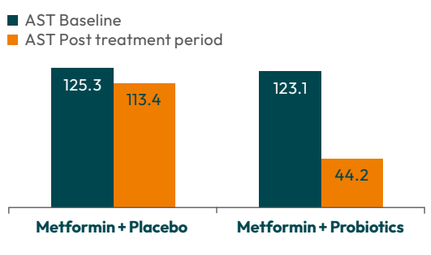
However, there was significantly greater improvement in the probiotic group with the main results summarised below:

Effects on liver enzymes:

Effects of treatment on ALT (p < 0.001) (IU/L)

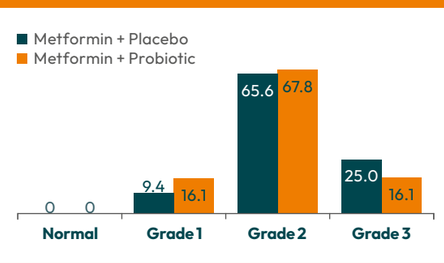


Effects of treatment on AST (p < 0.001) (IU/L)



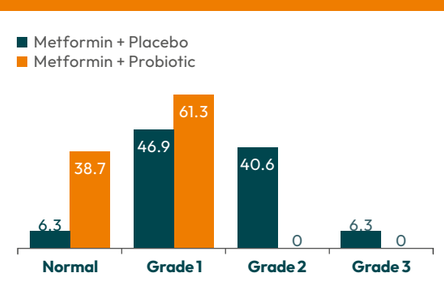
Effects on ultrasound grading of NASH

Pre-treatment ultrasound graded steatosis (% of patients)



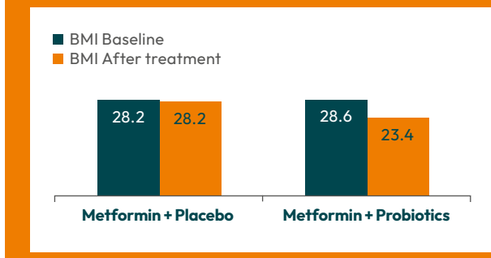
Above figure shows ultrasound graded staetosis before intervention. None of the patients had a normal grade ultrasound.

Post-treatment ultrasound graded steatosis (% of patients)



This figure shows the ultrasound grade steatosis after six months of treatment. 38.7% of patients had a normal grade ultrasound in the metformin/probiotic group; 61.3% patients had grade 1 ultrasound appearances and none of the patients had grade 2 or 3. The differences were statistically significant as compared to the Metformin/placebo group (P=0.01).

Changes in BMI after treatment (p < 0.001) (kg/m^2)



The group taking the probiotic supplement alongside conventional management was found to have a significantly reduced BMI (p = 0.001) whilst the group taking placebo did not show any changes.

Conclusion

This study suggests that the Protexin probiotic formulation helps to improve the efficacy of standard management of NASH with a number of benefits including weight loss. The mechanism of action is not fully understood, but the evidence supports the use of probiotics in the treatment of NASH and certainly supports further study to establish the exact pathways that are being influenced.

Shavakhi A, Minakari M, Firouzian H, Assali R, Hekmatdoost A, Ferns G. Effect of a Probiotic and Metformin on Liver Aminotransferases in Non-alcoholic Steatohepatitis: A Double Blind Randomized Clinical Trial. *Int J Prev Med* 2013;**4**:531–7.

The clinical efficacy of probiotics in the treatment of **functional constipation** in men, RCT

Protexin probiotics significantly reduced abdominal cramps and constipation

Objective

To evaluate the effects of a Protexin probiotic formulation on functional constipation in males.

The Protexin multi-strain formulation
Lactobacillus casei PXN 37
Lactobacillus rhamnosus PXN 54
Streptococcus thermophilus PXN 66
Bifidobacterium breve PXN 25
Lactobacillus acidophilus PXN 35
Bifidobacterium longum PXN 30
Lactobacillus bulgaricus PXN 39
Fructooligosaccharide (FOS)
Total viable count per capsule 1 x 10^8 CFU
Daily dose: 2 capsules (2 x 10^8 CFU)

Methods

A randomised, placebo controlled trial in 60 men suffering with functional constipation (FC). The men were randomised to receive either the probiotic capsule or the placebo capsule. Both capsules were identical and participants took one capsule twice a day for 4 weeks. Outcome measures were number of bowel movements (BMs) per week, the completion of a Patient Assessment of Constipation Symptoms Questionnaire (PAC-SYM) and Bristol stool form scale.

Results

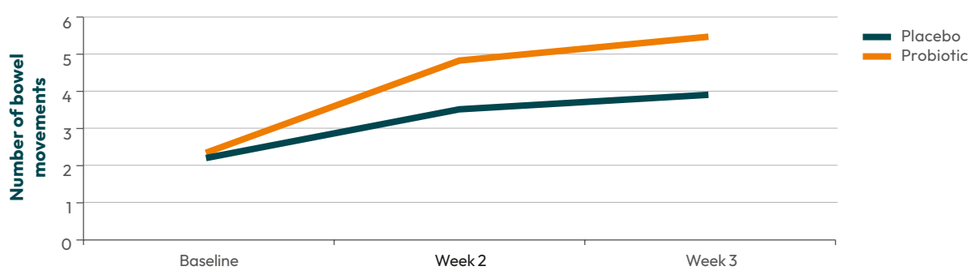
There was a significance increase in bowel movements in the probiotic group compared to the placebo at weeks two and four. BMs increased from 2.29 / week in the probiotic group to 4.81 at week two and to 5.45 BMs in week four ($p = 0.02$).

There was also a significant difference seen at weeks two and four in the Bristol stool form scale ($p = 0.0006$), as well as improvements in the PAC-SYM, namely reduction in stomach cramps ($p = 0.02$) and reduction in the frequency of BMs being too small ($p = 0.03$).

Conclusion

This study shows that the Protexin probiotic formulation is able to significantly increase bowel movements per week as well as reducing abdominal cramps and frequency of small bowel movements in constipation sufferers. There were no side effects reported with the consumption of the probiotic in this study.

The effect of probiotic on average number of bowel movements per week in FC sufferers



Fateh R, Iravani S, Frootan M, Rasouli MR, Saadat S. Synbiotic preparation in men suffering from functional constipation: a randomised controlled trial. *Swiss Med Wkly* 2011;**141**:w13239.

The clinical efficacy of probiotics as an adjunct in the treatment of **bacterial vaginosis**, RCT

Protexin probiotics significantly reduced bacterial vaginosis

Objective

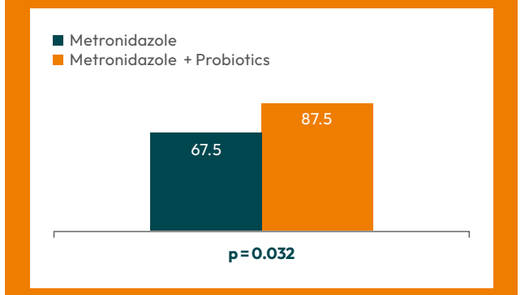
To compare the efficacy of metronidazole versus a combination of metronidazole and a Protexin probiotic formulation in the treatment of bacterial vaginosis.

The Protexin multi-strain formulation
Lactobacillus casei PXN 37
Lactobacillus rhamnosus PXN 54
Streptococcus thermophilus PXN 66
Bifidobacterium breve PXN 25
Lactobacillus acidophilus PXN 35
Bifidobacterium longum PXN 30
Lactobacillus bulgaricus PXN 39
Vitamin C
Vitamin A
Vitamin E
Fructooligosaccharide (FOS)
Total viable count per capsule 1 x 10^8 CFU
Daily dose: 2 capsules (2x10^8 CFU)

Methods

80 women were included in the clinical trial. Bacterial vaginosis was diagnosed using Amsel criteria. All patients were given standard treatment (metronidazole) and half of the cohort was randomised to receive probiotic as a complementary treatment. Patients were assessed for symptoms and Amsel criteria at baseline and at 3-7 days post treatment. Treatment success was defined as the absence of any of Amsel's criteria.

Treatment success (%)



Results

There was significant improvement in symptoms in both groups. Symptoms of vaginal discharge, itching, and foul smelling discharge improved in all patients. However, the probiotic group showed a higher rate of treatment success (87.5%) versus placebo (67.5%) and this was statistically significant ($p = 0.032$).

Conclusion

The inclusion of the Protexin probiotic formulation greatly increased the efficacy of treatment in this trial suggesting that it can be an effective adjunct to conventional treatment in the management of bacterial vaginosis.

Tafazzoli-harandi H, Akbari SAAA, Afrakhteh M, *et al.* Comparison of metronidazole versus a combination of metronidazole plus probiotics in the treatment of bacterial vaginosis. *J Women's Heal Issues Care* 2014;**3**:1000146.

The clinical efficacy of probiotics in **chronic** and **episodic migraine**, RCT

Objective

To determine the effects of a Protexin probiotic formulation on the duration, frequency and severity of migraine attacks in adults with chronic or episodic migraines.

The Protexin multi-strain formulation
Bacillus subtilis PXN 21
Bifidobacterium bifidum PXN 23
Bifidobacterium breve PXN 25
Bifidobacterium infantis PXN 27
Bifidobacterium longum PXN 30
Lactobacillus acidophilus PXN 35
Lactobacillus delbrueckii ssp. *bulgaricus* PXN 39
Lactobacillus casei PXN 37
Lactobacillus plantarum PXN 47
Lactobacillus rhamnosus PXN 54
Lactobacillus helveticus PXN 45
Lactobacillus salivarius PXN 57
Lactococcus lactis ssp. *lactis* PXN 63
Streptococcus thermophilus PXN 66
Total viable count per capsule 2 x 10⁹ CFU
Daily dose: 2 capsules (4 x 10⁹ CFU)

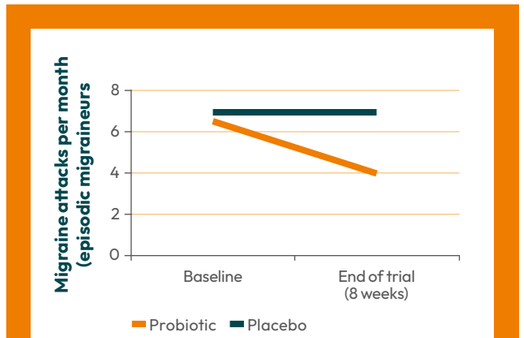
Methods

This was a randomised, double blind, placebo controlled prospective study with 500 participants screened for migraines. 50 patients diagnosed with chronic migraines (ICHD-II) and 50 patients diagnosed with episodic migraines (ICHD-IIIβ) were enrolled. Each group was further divided into probiotic and placebo arms. Groups received two capsules of probiotics or placebo per day, for 8 weeks (chronic migraine) or 10 weeks (episodic migraine). Regular prophylactic medications were used as usual. A 30-days headache diary was used to monitor frequency, intensity and duration of migraine attacks as well as use of abortive drugs. Migraine disability assessment scale (MIDAS) was also used. Serum inflammation markers (CRP, TNFα) were measured.

Reduction of headache markers in probiotic groups when compared to placebo

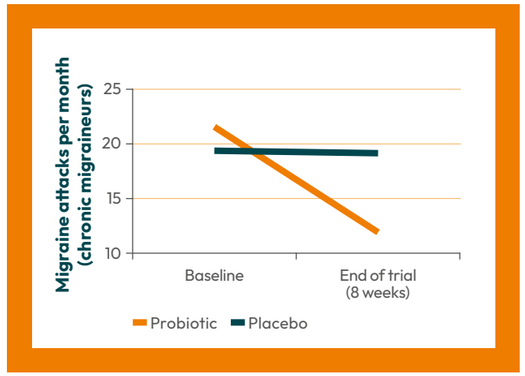
Outcome	Chronic migraine	Episodic migraine
Abortive drugs used	38%***	35%***
Headache frequency	45%***	40%***
Headache severity	31%***	29%***
Headache duration	36min*	15min
MIDAS score	26%*	30%***

*p<0.05, ***p<0.001



Results

All migraine markers were improved in both chronic and episodic migraine groups taking probiotics, compared to placebo, but CRP and TNFα levels were similar between the groups. Reduction of headache frequency and severity significantly reduced disability score in both chronic and episodic migraine patients.



Conclusions

Protexin probiotic formulation achieved a significant reduction in the frequency and severity of migraine headaches severity in both chronic and episodic migraine sufferers. This study suggests that using Protexin probiotic alongside current prophylactic medications would have beneficial effects on migraine control.

Use of Protexin probiotics reduced frequency of migraine attacks by over 40% in both chronic and episodic sufferers

Martami F, Togha M, Seifishahpar M, *et al.* The effects of a multispecies probiotic supplement on inflammatory markers and episodic and chronic migraine characteristics: A randomized double-blind controlled trial. *Cephalalgia.* Jan 2019.

Martami F, Seyfi-shahpar M, Ghorbani Z, Jahromi SR, Togha M, Ansari H. Multi-species probiotic mixture can attenuate the severity of episodic migraine- a double blind randomized controlled trial. *Cephalalgia* 2017;**37**:369-370.

Seyfi-Shahpar M, Martami F, Togha M, Ghorbani Z, Jahromi SR, Ansari H. The Effect of probiotic supplementation on chronic migraine (CM) headache: a randomized placebo-controlled double blind study. *Journal of Headache and Pain* 2017;**18**:159.

The clinical efficacy of probiotics in diarrhoea predominant **irritable bowel syndrome**, RCT

Use of Protexin probiotics in IBS-D patients reduced abdominal pain and distention within first 4 weeks, and continued to show improvements for the duration of the study

Objective

To assess the effect of a Protexin probiotic formulation on gastrointestinal symptoms and quality of life in adults with diarrhoea-predominant irritable bowel syndrome (IBS-D).

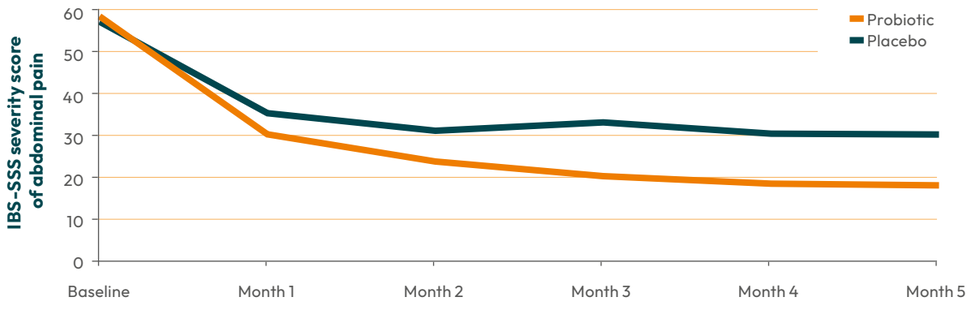
The Protexin multi-strain formulation
Bacillus subtilis PXN 21
Bifidobacterium bifidum PXN 23
Bifidobacterium breve PXN 25
Bifidobacterium infantis PXN 27
Bifidobacterium longum PXN 30
Lactobacillus acidophilus PXN 35
Lactobacillus delbrueckii ssp. *bulgaricus* PXN 39
Lactobacillus casei PXN 37
Lactobacillus plantarum PXN 47
Lactobacillus rhamnosus PXN 54
Lactobacillus helveticus PXN 45
Lactobacillus salivarius PXN 57
Lactococcus lactis ssp. *lactis* PXN 63
Streptococcus thermophilus PXN 66
Total viable count per capsule 2 x 10^9 CFU
Daily dose: 4 capsules (8 x 10^9 CFU)

Methods

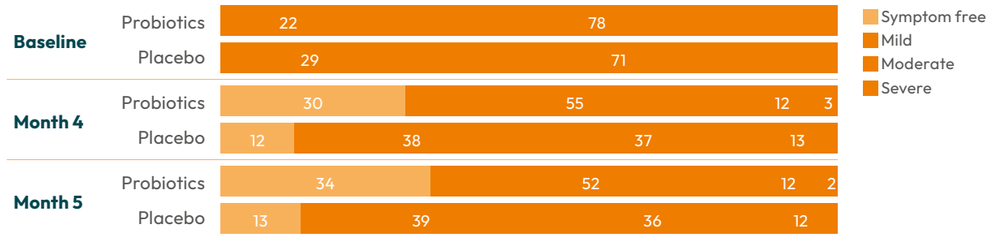
In this randomised, double blind, placebo controlled trial 400 moderate to severe symptomatic IBS-D patients, diagnosed by Rome III criteria, were enrolled. They were given either probiotics or placebo for 16 weeks and were followed up for another 4 weeks after the end of the intervention. The IBS symptoms (abdominal pain and frequency) were measured with IBS-SSS questionnaire (max 500 points) and quality of life (QoL) was measured by the 34-item IBS-QoL questionnaire every month. No change in lifestyle/diet was introduced.

Results

181 patients in the probiotic group and 179 in the placebo group completed the trial. At month 5 abdominal pain level had decreased by almost 70% in the probiotic group vs 47% in the placebo group ($p<0.001$). The overall IBS-SSS in the probiotic group was reduced by 145 points within 30 days (vs. 117 points in the placebo group) and by 223 points by month 5 (vs. 157 points in the placebo group). IBS pain frequency decreased by over 70% in the same time in the probiotic group (from 7.7 to 2.2 per 10 days), which was a 20% greater reduction than in the placebo group (from 8.1 to 3.9 per 10 days).

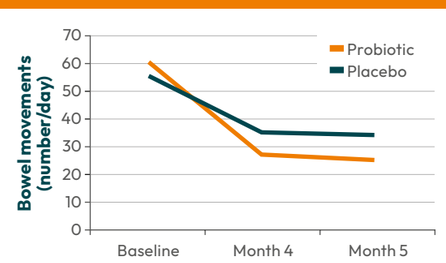


Proportion of IBS severity groups at baseline, month 4 and month 5 in probiotics and placebo groups (%)



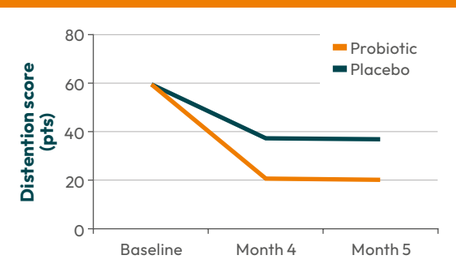
Significant improvement in abdominal pain level ($p=0.002$) and frequency ($p=0.001$), as well as distention (0.028) was seen within the first month of the trial. The number of bowel motions per day was significantly reduced from month 2 onwards in the probiotic group, compared with the placebo group ($p=0.043$ at month 2).

Change in bowel motions between baseline and end of trial



At baseline, all patients rated their symptoms as moderate to severe while at the end of the trial, this was reduced to 13.8% in the probiotic group compared with 48.0% in the placebo group ($p<0.001$). At the end of the trial 34% of patients in the intervention group vs 13% in placebo group were symptom-free.

Change in distention score in IBS-SSS between baseline and end of trial



All dimensions of QoL showed significantly greater and consistent improvement in the intervention group than in the placebo group. No serious adverse events were reported.

Conclusions

Protexin probiotic formulation was associated with a statistically significant consistent improvement in overall symptom severity, including pain, distention and bowel movements, in patients with IBS-D, and was well tolerated.

Ishaque SM, Khosruzzaman SM, Ahmed DS, Sah MP. A randomized placebo-controlled clinical trial of a multi-strain probiotic formulation (Bio-Kult®) in the management of diarrhea-predominant irritable bowel syndrome. *BMC Gastroenterol* 2018;**18**:71

The clinical efficacy of a combined probiotic-cranberry extract formulation in preventing recurrent **urinary tract infections**, RCT

Objective

To assess the effect of an Protexin probiotic-cranberry extract formulation in the prevention of recurrent urinary tract infections (rUTI).

The Protexin multi-strain formulation
Lactobacillus acidophilus PXN 35
Lactobacillus plantarum PXN 47
Cranberry Extract
Vitamin A
Total viable count per capsule 5 x 10^8 CFU
Daily dose: 2 capsules (1 x 10^9 CFU)

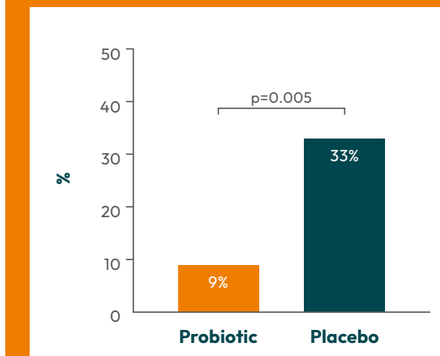
Methods

In this randomised, double blind, placebo controlled trial 90 adult pre-menopausal women suffering with rUTI (≥2 episodes of uncomplicated acute infections in the last 6 months, or ≥3 episodes in the last 12 months) were enrolled. They were given either a probiotic-cranberry extract combination product or placebo daily for 6 months. As per European Association of Urology (EAU) guidelines uncomplicated UTI was diagnosed by >10^3 CFU/ml of uropathogens in a mid-stream sample of urine in participants presenting with typical UTI symptoms (painful urination, urinary frequency, urinary urgency, low abdominal pain, and bloody urine).

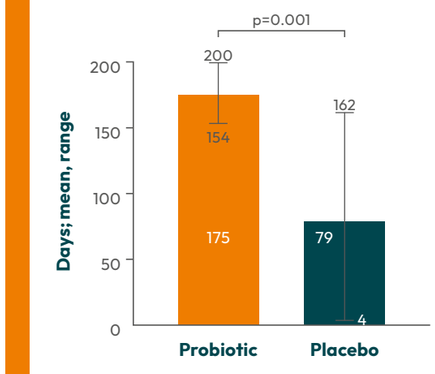
Results

81 (90%) of the 90 patients completed the study. During the 6 month intervention 33% of patients in the placebo group experienced a UTI episode, compared to only 9% in the probiotic group (p<0.01); equating to a 73% reduction in the rate of infections. In the probiotic group none of the participants experienced >1 UTI episode during the study compared to 4 in placebo group. The time to first UTI episode in the probiotic group was on average 96 days longer than in the placebo group (175 vs 79 days; P = 0.001), and the duration of UTI episodes were on average 7 days shorter in the probiotic group (5 vs 12 days; P = 0.009). 72% less subjects in the probiotic group required treatment with antibiotics (3 vs 11; P = < 0.05), the placebo group were exposed to significantly more antibiotic courses (14 vs 3; p = <0.05), and the duration of antibiotic treatment in the probiotic group was nearly halved compared to the placebo group (4 vs 7 days; P = 0.09).

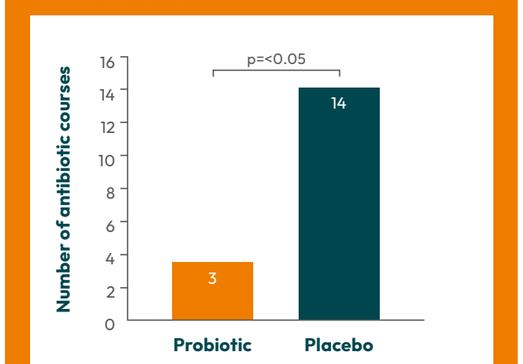
Participants with RUTI in follow up



Time to first UTI



Antibiotic courses



Conclusion

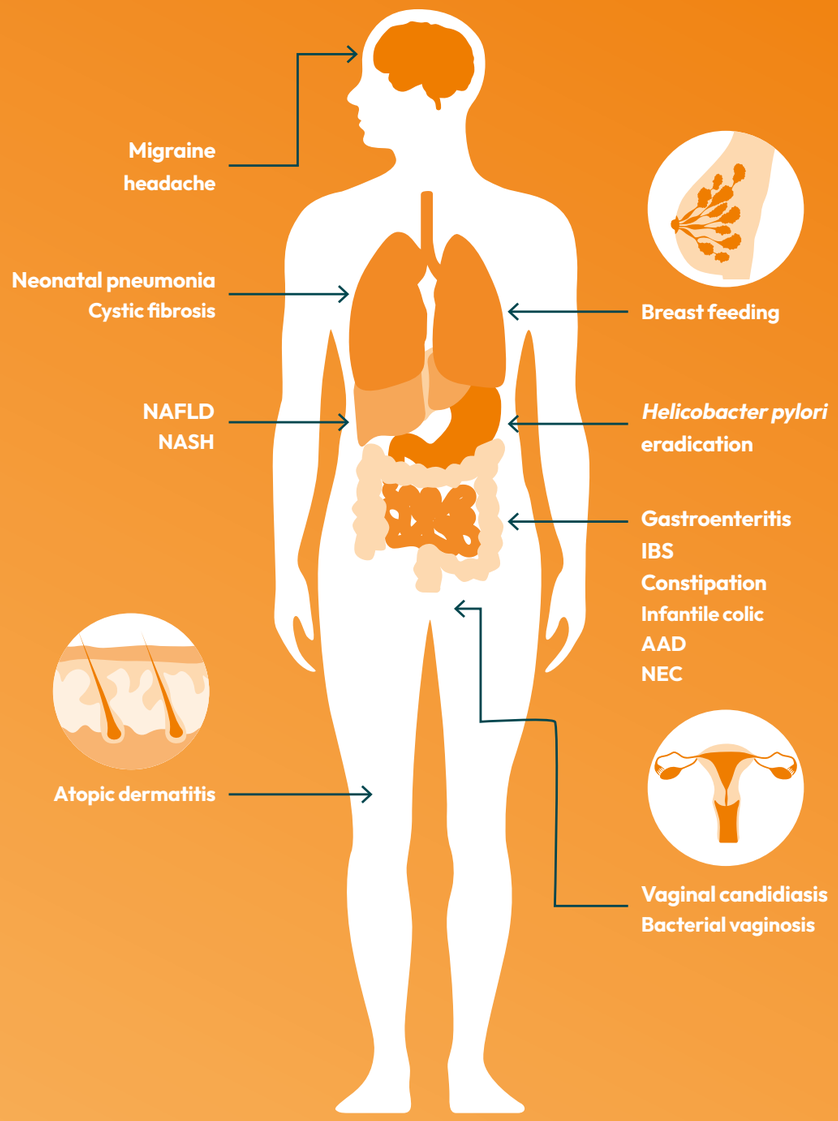
The Protexin probiotic-cranberry supplement achieved a significant reduction in recurrent UTIs and reduced the antibiotic exposure in adult women during a 6 month period.

Protexin probiotics significantly reduce the recurrence of urinary tract infections in adult women

Koradia P, Kapadia S, Trivedi Y, Chanchu G, Harper A. Probiotic and cranberry supplementation for preventing recurrent uncomplicated urinary tract infections in premenopausal women: a controlled pilot study. *Expert Review of Anti-infective Therapy* 2019 (DOI: 10.1080/14787210.2019.1664287)

Summary of **systematic reviews**

Over the last decade, our research has contributed to building strong evidence for probiotics in various conditions. Our research publications have been referenced over 200 times, and many trials have been included in systematic reviews, including Cochrane reviews. The importance of probiotics in preventing antibiotic associated diarrhoea has been recognised by important bodies like the National Institute for Health and Care Excellence Clinical Knowledge Summaries, who in March 2018 recommended using probiotics in all patients using antibiotics (children and adults as well as inpatients and outpatients).



Protexin trials in systematic reviews

CYSTIC FYBROSIS

Protexin formulations have been shown to be effective in reducing pulmonary exacerbations in CF patients and demonstrated the highest reduction in faecal calprotectin levels, when compared to other probiotic studies included in systematic reviews.

- Coffey MJ, Garg M, Homaira N, Jaffe A, Ooi CY. Probiotics for people with cystic fibrosis. *Cochrane Database Syst Rev* 2018;**2**.
- Ananthan A, Balasubramanian H, Rao S, Patole S. Probiotic supplementation in children with cystic fibrosis—a systematic review. *Eur J Pediatr* 2016;**175**:1255-1266.
- Nikniaz Z, Nikniaz L, Bilan N, Somi MH, Faramarzi E. Does probiotic supplementation affect pulmonary exacerbation and intestinal inflammation in cystic fibrosis: a systematic review of randomized clinical trials. *World J Pediatr* 2017;**13**:307-13.

H.PYLORI AND AAD

Protexin formulation has been shown to increase H. pylori eradication rate more than the average effect of probiotics shown in systematic reviews (by 30% vs 13%). In addition, it has shown to significantly reduce side effects of antibiotic treatment by over 67%, which was greater than the average probiotic effect of 41%.

- Feng J-R, Wang F, Qiu X, *et al.* Efficacy and safety of probiotic-supplemented triple therapy for eradication of Helicobacter pylori in children: a systematic review and network meta-analysis. *Eur J Clin Pharmacol* 2017;**73**:1199-208.
- Li S, Huang X, Sui J, *et al.* Meta-analysis of randomized controlled trials on the efficacy of probiotics in Helicobacter pylori eradication therapy in children. *Eur J Pediatr* 2014;**173**:153-61.
- Zhang M-M, Qian W, Qin Y-Y, He J, Zhou Y-H. Probiotics in Helicobacter pylori eradication therapy: a systematic review and meta-analysis. *World J Gastroenterol* 2015;**21**:4345-57.

OBESITY & BLOOD GLUCOSE

Protexin formulations were shown along with other multispecies formulations, to be effective in reducing fasting blood glucose.

- Kobyliak N, Conte C, Cammarota G, *et al.* Probiotics in prevention and treatment of obesity: a critical view. *Nutr Metab (Lond).* 2016;**13**:14.
- Nikbakht E, Khalesi S, Singh I, Williams LT, West NP, Colson N. Effect of probiotics and synbiotics on blood glucose: a systematic review and meta-analysis of controlled trials. *Eur J Nutr* 2018;**57**:95-106.
- Ruan Y, Sun J, He J, Chen F, Chen R, Chen H. Effect of Probiotics on Glycemic Control: A Systematic Review and Meta-Analysis of Randomized, Controlled Trials. *PLoS One* 2015;**10**:e0132121.

INFANT COLIC

Protexin formulation was effective in reducing cry and fuss time significantly more than placebo, with an average reduction of 35 minutes. It was shown to have a markedly better effect than LGG (overall no reduction) and L. reuteri which showed inconsistent results between the studies and lack of effect in the largest study using L.reuteri.

- Harb T, Matsuyama M, David M, Hill RJ. Infant Colic–what works: a systematic review of interventions for breast-fed infants. *Journal of pediatric gastroenterology and nutrition* 2016;**62**:668-86.
- Szajewska H, Dryl R. Probiotics for the Management of Infantile Colic. *J Pediatr Gastroenterol Nutr* 2016;**63**:S22-S24.
- Dryl R, Szajewska H. Probiotics for management of infantile colic: a systematic review of randomized controlled trials. *Arch Med Sci* 2018;**14**:1137-43.

LIVER FUNCTION

Markers of liver function have been significantly improved in individuals taking Protexin formulation. The magnitude of the effect was significantly greater than that achieved by other probiotics. This included two times greater weight loss than the second most effective probiotic, 3 fold higher reduction in ALT and AST than the second best probiotic and 5 times greater reduction in serum triglycerides than the second best probiotic.

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ATOPIC DERMATITIS

Use of Protexin formulation reduced Severity Scoring of Atopic Dermatitis (SCORAD) over three times more than the average effect of other probiotics (by 19 points compared to 6.5 points).

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Notes

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