Research Title: Probiotics for the prevention of Upper Respiratory Tract Infections (PROBINS study)

Background

In a double-blind placebo controlled trial, we have demonstrated the effectiveness of our Lab4 probiotic formulation in children (Garaiova *et al.*, 2014). Significant reductions in the incidence rate of upper respiratory tract infection (URTI; 33%, *P*=0.002), the number of days with URTI symptoms (mean difference: -21.0, 95% confidence interval (CI):-35.9, -6.0, *P*=0.006) and the incidence rate of absence from preschool (30%, *P*=0.007) were observed in the active group compared with the placebo. The number of days of use of antibiotics, painkillers, cough medicine or nasal sprays was lower in the active group and reached significance for use of cough medicine (mean difference: -6.6, 95% CI: -12.9, -0.3, P=0.040).

Probiotics have been shown to reduce the number of episodes of acute URTI, the mean duration of an episode of acute URTI, antibiotic use and cold-related absenteeism (Hao *et al.*, 2015), and it is known that some probiotic bacteria can stabilise the composition of the gut microbiota and can modulate/stimulate the immune response.

Studies with probiotics, other than Lab4, have shown reductions in the incidence rate and duration of URTI (Hao *et al.*, 2015 and Shida *et al.*, 2015). Bolyard *et al.,* 1998 showed that incidence rates for colds and flu were higher among working populations interacting directly with the public (e.g. teachers and healthcare workers). Absence from work linked to URTIs represents a considerable economic burden but there are also financial impacts associated with antimicrobial usage. The aim of this study is to assess the potential of the Lab4 consortium of probiotic bacteria to impact on URTI.

Hypothesis and objectives

Hypothesis: Daily supplementation with Lab4 probiotics will reduce the incidence and/or duration of Upper Respiratory Tract Infections (URTI), improving quality of life and wellbeing.

Objective: Double blind randomised placebo-controlled study to investigate the impact of 4 months' supplementation with Lab4 probiotics (50 billion colony forming units (CFU) per day) on the incidence and duration of upper respiratory infections in participants aged between 18-70 years.

Intervention

The participants will receive a daily capsule containing either 50 billion viable probiotic bacteria or an inert placebo. Each participant will be given their first supply of the probiotic or placebo at the start of the trial and will receive the second bottle of capsules at the mid-trial review meeting. Participant randomisation will be performed independently by a statistician.

Requirements from participants

Take a daily supplement for a period of 4 months, provide samples (blood, faeces, saliva and urine) and physiological measurements at the beginning and end of the trial and complete a daily update.

Product specification

The active product will contain the Lab4 probiotic consortium (*Lactobacillus acidophilus* CUL-60 (NCIMB 30157), *Lactobacillus acidophilus* CUL-21 (NCIMB 30156), *Bifidobacterium bifidum* CUL-20 (NCIMB 30153) and *Bifidobacterium lactis* CUL-34 (NCIMB 30172)) at a total of 5 x 10^{10} colony forming units per day. The placebo will be identical looking capsule containing microcrystalline cellulose and maltodextrin. Both interventions will be prepared by Cultech Ltd, Port Talbot, UK.

Proposed Inclusion criteria

- Healthy males or females aged 18 to 70 years

Proposed Exclusion criteria

- Participants who are unable to give written informed consent.
- Participants who are not prepared to provide faecal, blood, saliva or urine samples as required.
- Participants who are taking the products/medications that stimulate immune function/inflammation. For example: β glucans, isoprinosine (methisoprinolum), ribomunyl, immunomodulants lysate of bacteria
- Participants who have taken probiotic supplements within 2 weeks of trial start.

- Participants who are pregnant or lactating.
- Participants who have received oral antibiotics within 3 weeks of trial start.

Proposed Methodology

104 participants who work within a healthcare setting are to be recruited (stage 1) and asked to provide a stool sample (faeces), urine, saliva and a blood sample at baseline (stage 2), at the beginning and upon completion of the trial (stage 4) (Figure 1). At the start and end of the trial (stages 2 and 4), each participants' resting blood pressure and peak flow rate will also be measured. The incidence and duration of URTI symptoms and compliance to the intervention will be recorded by the participant via either a smart phone application or by completion of a daily symptom questionnaire. Participants will be asked to complete a brief questionnaire on health and wellbeing at the start middle and end of the trial (stages 2, 3 and 4).

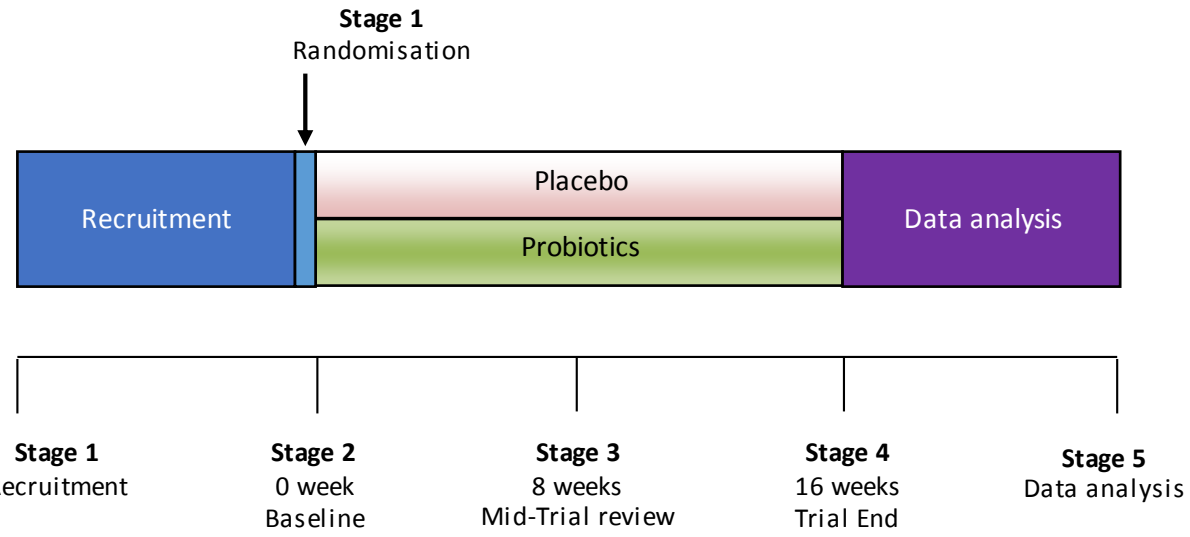

Figure 1-Diagramatic outline of study design. Stage 1, recruitment and randomisation of participants. Stage 2, Baseline measurements. Stage 3, Mid-trial review. Stage 4, Trial end and stage 5, data analysis.

Primary Outcomes

Incidence of URTI symptoms
Duration of URTI symptoms.

Secondary Outcomes

Numbers of participants experiencing episodes of URTI
Antibiotic usage
Absence from work relating to URTI
Number of GP visits relating to URTI
Antibiotic usage

Analysis of faecal bacterial populations
Analysis of health-related biomarkers in blood/urine/saliva
Quality of life

Results to be processed

- Symptom questionnaires
- Blood pressure: Recorded at beginning and end of intervention.
- Body weight: Recorded at beginning and end of intervention.
- Peak flow: Recorded at beginning and end of intervention
- Faecal samples: DNA extraction and microbial populations analysed by deep sequencing (MiSeq) alongside traditional microbiological analysis
- Blood: Serum inflammatory cytokine profiles to be assessed using MSD platform and lipid profiles (Triglycerides, Total Cholesterol, LDL and HDL) to be assessed
- Urine: cryo-banked for future analysis.
- Saliva: cryo-banked for future analysis.

Sample requirements

Table 1- Samples to be provided by participants

Sample	Stage required	Total number of samples
Symptom questionnaires	Completed daily	All participants
Physiological measurements	2 and 4	All participants
Quality of life questionnaires	2, 3 and 4	All participants
Stool	2 and 4	Minimum of 40 participants
Blood	2 and 4	All participants
Urine	2 and 4	Minimum 40 participants
Saliva	2 and 4	All participants

- Stool sample: Stages 2 and 4
- Blood sample: Stages 2 and 4
- Urine sample: Stages 2 and 4
- Saliva sample: Stages 2 and 4
- Physiological data collection: stages 2, 3 and 4
- Quality of life questionnaires: stage 2, 3 and 4
- Symptom questionnaires: repeated daily

Safety

The Lab4 probiotic consortium has been on sale in the UK, USA and Canada under various brand names since 1996.

The Lab4 bacterial strains have been used in a number of previous studies and no adverse events have been recorded:

1. Tazzyman S, Richards N, Trueman AR, Evans AL, Grant VA, Garaiova I, Plummer SF, Williams EA, Corfe B. Vitamin D associates with improved quality of life in participants with irritable bowel syndrome: outcomes from a pilot trial. BMJ Open Gastro 2015;2:e000052. doi:10.1136/bmj gast-2015- 00005
2. Garaiova I, Muchová J, Nagyová Z, Wang D, Li JV, Országhová Z, Michael DR, Plummer SF, Ďuračková Z. Probiotics and vitamin C for the prevention of respiratory tract infections in children attending preschool: a randomized controlled study**.** European Journal of Clinical Nutrition 2015
3. Williams E, Simpson J, Wang D, Plummer S, Garaiova I, Barker M, Corfe B. Clinical trial: a multistrain probiotic preparation significantly reduces symptoms of irritable bowel syndrome in a double-blind placebo-controlled study. Alimentary Pharmacology and Therapeutics 2009, 29:97-103.
4. Plummer S, Garaiova I, Sarvotham T, Cottrell S, Le Scouiller S, Weaver M, Tang J, Dee P, Hunter J. Effects of Probiotics on the composition of the intestinal microbiota following antibiotic therapy. International Journal of Antimicrobial Agents 2005, 26: 69-74.
5. Madden J, Plummer S, Tang J, Garaiova I, Plummer N, Herbison M, Hunter J, Shimada T, Cheng L, Shirakawa T. Effect of Probiotics on preventing disruption of the intestinal microflora following antibiotic therapy: A double blind, placebo controlled pilot study. International Immunopharmacology 2005, 5: 1091-1097.
6. Plummer S, Weaver M, Harris J, Dee P, Hunter J. *Clostridium difficile* pilot study: effects of probiotic supplementation on the incidence of *C. difficile* diarrhoea. International Microbiology 2004 7:59-62.

References

Garaiova *et al* (2014), Probiotics and vitamin C for the prevention of respiratory tract infections in children attending preschool: a randomised controlled pilot study. European Journal Clinical Nutrition. 69(3): 373–379.

Hao *et al* (2015), Probiotics for preventing acute upper respiratory tract infections, Cochrane Acute Respiratory Infection Group.

Shida *et al* (2015), Daily intake of fermented milk with *Lactobacillus casei* strain Shirota reduces the incidence and duration of upper respiratory tract infections in healthy middle-aged office workers, European Journal of Clinical Nutrition, DOI 10.1007/s00394-0151056-1

Bolyard *et al* (1998), Guideline for infection control in health care personnel, American Journal of Infection Control, 26:289-354.