The Effects of Probiotics on Symptoms of Depression: Protocol for a Double-Blind Randomized Placebo-Controlled Trial

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Keywords

Depression · Probiotics · Gut-brain axis · Microbiome · Clinical trial · Protocol

Abstract

Background: A growing body of evidence has linked mental health outcomes to the gut microbiome. This has led to the investigation of the GI tract as a target for novel treatments and interventions for depression, including probiotic supplementation. Our recent pilot study provided the first evidence of probiotics improving symptoms of depression in treatment-naïve depressed patients. To further support and expand upon this evidence, data from the pilot study were used to plan a 16-week, double-blind, randomized, placebo-controlled trial to assess the effects of probiotics on depression. Here, we report the protocol for this trial. **Methods:** Participants diagnosed with depression will orally consume a probiotic supplement containing *Lactobacillus helveticus* and *Bifidobacterium longum* or placebo once daily. Participants will undergo assessments measuring clinical outcomes using a battery of validated clinical scales and questionnaires. Sleep architecture and quality will be measured using polysomnography. Neuroimaging data will be collect-

ed using magnetic resonance imaging to examine functional and structural neurophysiological changes. Molecular data will be collected from blood, stool, and urine samples to examine cytokine levels and explore potential genes and proteins that may predict outcomes in depression. **Results:** We expect results to replicate and expand on our pilot data demonstrating that probiotics may be effective in alleviating symptoms of depression, and to find biomarkers that will predict these outcomes. **Conclusions:** The findings from this study will add to the growing body of research in this emerging field, which eventually may provide evidence for probiotics having a role in alleviating symptoms of depression.

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Introduction

Major depressive disorder (MDD) is a devastating yet widespread chronic disorder with considerable personal and socioeconomic costs. According to the World Health Organization, MDD is the fourth leading cause of disability worldwide, and it will be the second leading cause by 2020 [1]. Individuals suffering from MDD experience persistent low mood, blunted or absent experience of

pleasure, as well as a wide range of cognitive, physical, and behavioral symptoms that interfere with the individual's capacity to function in daily life. First-line pharmacological treatments for MDD have an increasingly high failure rate [2]; thus, identifying new treatment targets and therapies is very important.

Previously, it was generally accepted among clinicians and scientists that MDD was a disorder of reduced monoaminergic activity in the brain [3]. Consequently, most pharmacological treatments currently available solely target these neurotransmitters and their receptors. Others have postulated glutamate receptor involvement (e.g., with the use of the N-methyl-D-aspartic acid [NMDA] receptor agonist ketamine) or the modulation of cholinergic and γ -aminobutyric acid (GABA)ergic transmission [4]. Treatments targeting these systems may alleviate symptoms of depression by directly modulating neurotransmitter function in the brain. The pathophysiology of MDD, however, is far more complex, extending beyond correcting the balance of certain neurotransmitters. Other systems, including the immune system and its role in neuroinflammation, have been implicated in several mental illnesses including MDD [5]. Thus, modulators of host immunity are currently being investigated as novel treatment targets for mental illness [5].

A growing body of evidence has linked gut microbiome composition and activity to central nervous system development and functioning [7–9]. Referred to as the gut-brain axis, this bidirectional communication network between the brain and the gut has emerged as a potential target for novel antidepressant treatments. Research has demonstrated that the microbiome of depressed patients differs in terms of composition and diversity [10]. Accordingly, preclinical and clinical research has implicated probiotics – bacteria in the gastrointestinal (GI) tract that can confer a health benefit on the host [11] – in improving symptoms of depression in both rodents [12–14] and healthy humans [15–19]. Several studies have reported probiotic-induced brain changes in areas associated with depression [19, 20] though research on clinical samples of patients with depression remains scant with inconsistent findings [20–22].

Probiotics may be advantageous because they may target and influence the underlying inflammatory pathophysiology of the disorder. It is possible that the effects of probiotics on psychiatric symptoms are mediated through the gut-brain axis by decreasing systemic inflammation and modulating neurotransmission [23]. By maintaining the integrity of the GI lining, probiotics reduce permeability caused by compromised tight junctions of endothe-

lial cells in the GI lining, thus impeding bacteria and toxins such as lipopolysaccharide (LPS) to leak from the GI tract into the bloodstream. LPS, an endotoxin present in the membrane of gram-negative bacteria, induces an elevated immune response including a release of proinflammatory cytokines, which is a common phenotype seen in depressed patients [24]. These proinflammatory cytokines can travel across circumventricular organs to the brain, affecting neurotransmission [25] by disrupting metabolic pathways from tryptophan to kynurenine [26], and resulting in depressive symptoms such as fatigue, sleep disturbances, reduced appetite, irritability, and depressed mood [27].

Our recent systematic review of the effects of probiotics on symptoms of depression in humans revealed limited but promising preliminary research in this field [28], but exposed many gaps and inconsistencies in the research published to date, including the variability in specific strains of probiotics being studied, the duration of the intervention, and the dosage. Although 7 of the 10 included studies reported positive findings, the review highlighted a critical need for more blinded, randomized, placebo-controlled trials in clinical populations to elicit any findings confirming positive effects. A recent meta-analysis also demonstrated promise for probiotics in alleviating depressive symptoms but emphasized the need for further studies in clinical populations [29].

Thus, we conducted an open-label pilot study assessing the efficacy, safety, and tolerability of a probiotic supplement on symptoms of depression in 10 treatment-naïve, depressed patients. Participants recruited from the Kingston area diagnosed with MDD and a Montgomery-Åsberg Depression Rating Scale (MADRS) [30] score ≥ 20 consumed Probio'Stick® daily at a dose of 3×10^9 CFU for 8 weeks. Throughout the 8 weeks, we measured subjective mood using the MADRS and Quick Inventory of Depressive Symptoms 16-Item Self-Report Questionnaire (QIDS-SR16) [31], anhedonia using the Snaith-Hamilton Pleasure Scale (SHAPS) [32], anxiety using the Generalized Anxiety Disorder 7-Item Scale (GAD-7) [33] and the State Trait Anxiety Inventory (STAI) [34], coping using the Ways of Coping Questionnaire (WCQ) [35], and sleep quality using the Pittsburgh Sleep Quality Index (PSQI) [36], in addition to objectively measuring sleep quality with a polysomnogram. The final sample was 70% female and had an average age of 25.2 years. Results from the pilot study showed significant improvements in mood, anhedonia, anxiety, and subjective sleep quality (see Table 1). Objective sleep data, however, showed no significant improvements in any of the measures of inter-

Table 1. Descriptive statistics of the pilot study (means $\pm$ SD)

Measure	Baseline	Week 4	Week 8	<i>p</i>
<i>Clinical</i>				
MADRS	24.9 $\pm$ 3.5	15.4 $\pm$ 4.4	12.7 $\pm$ 8.1	0.000
QIDS-SR16	20.5 $\pm$ 6.4	14 $\pm$ 6.3	11.6 $\pm$ 8.1	0.000
SHAPS	36.8 $\pm$ 2	31.4 $\pm$ 4.6	30.7 $\pm$ 6.8	0.001
GAD-7	13.5 $\pm$ 3.8	9.6 $\pm$ 4.9	9.8 $\pm$ 6.6	0.031
STAI	123.3 $\pm$ 11.3	105.7 $\pm$ 21.5	104.3 $\pm$ 28.1	0.008
WCQ	57.9 $\pm$ 15.9	64.4 $\pm$ 20.1	67.3 $\pm$ 27.8	0.223
PSQI	9.8 $\pm$ 3.6	8.6 $\pm$ 2.9	6.6 $\pm$ 2.8	0.005
<i>PSG</i>				
Sleep efficiency	89.3 $\pm$ 8.3	92.9 $\pm$ 5.9	92.9 $\pm$ 3.3	0.374
Sleep time in REM	21.6 $\pm$ 3.9	24.4 $\pm$ 8.4	23.7 $\pm$ 7.7	0.547
REM latency	67.4 $\pm$ 40.1	67.1 $\pm$ 25.6	85.2 $\pm$ 47.8	0.472
Sleep continuity	15.1 $\pm$ 6.5	16.1 $\pm$ 5.9	17 $\pm$ 6.2	0.706

est: sleep efficiency, time spent in REM sleep, REM latency, and sleep continuity. No significant improvements were seen in coping. Results for biological measures are not yet available.

These preliminary findings indicate a role for probiotics in alleviating depressive symptoms in mild-to-moderately depressed, treatment-naïve adults. Our objective for the present study is to expand upon these results by conducting a blinded, randomized, placebo-controlled trial assessing the effects of a probiotic supplement on symptoms of depression. The protocol was designed to align with other studies under the Canadian Biomarker Integration Network in Depression (CAN-BIND) umbrella [37]. Here, we describe the protocol for the trial.

Methods

Study Design

This is a 16-week, blinded, randomized, placebo-controlled dual-phase trial comparing Probio'Stick[®] to placebo in adults with MDD. At baseline, participants will be randomized into the Probio'Stick[®] arm or placebo arm. Participants in the Probio'Stick[®] arm will consume the investigational product (IP) daily for 8 weeks at a dose of 6×10^9 colony forming units (CFU); participants in the placebo arm will consume one identically looking sachet of placebo daily for 8 weeks (phase I). At week 8, participants will be assessed for treatment response. Probio'Stick[®] responders (defined as achieving a 50% or greater reduction in MADRS scores from baseline to week 8) will remain on the same dose as phase I, while non-responders will receive an increased dose of 20×10^9 CFU per day for the subsequent 8 weeks of the study (phase II). Placebo responders will continue in phase II with the same placebo dose as phase I while placebo non-responders will begin taking Probio'Stick[®] at the initial dose of 6×10^9 CFU per day (Fig. 1).

Table 2. Inclusion criteria

- 1 Diagnosis of major depressive disorder (MDD) according to DSM-IV determined by MINI
- 2 Current depressive episode with a MADRS score 20
- 3 Males and females aged 18–65 years
- 4 Able to understand and comply with the study requirements
- 5 Provision of written informed consent

Setting

The study will be carried out in a tertiary-care mental health and continuing care hospital in Kingston, ON, Canada. Polysomnography (PSG) will take place in participants' homes, and brain scans will take place at the Queen's University MRI Facility in Kingston, ON, Canada.

Participants

Eligible participants will be males and females aged 18–65 years diagnosed with MDD according to the criteria of the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) using the Mini-International Neuropsychiatric Interview (MINI). Eligible participants will be in a current episode of depression, have had fewer than 3 episodes of depression, are not currently taking any antidepressant medication, and have not failed a treatment during the current episode. Receiving regular psychotherapy is not an exclusion criterion for the study, but this information will be recorded (see Tables 2, 3 for full inclusion and exclusion criteria).

Investigational Product

Probio'Stick[®], developed and provided to the study by Lallemand Health Solutions (LHS), is a probiotic formulation containing 2 strains of probiotics, *Lactobacillus helveticus* R0052 and *Bifidobacterium longum* R0175 – deposited at the Institut Pasteur Collection Nationale de Cultures de Microorganismes as I-1722 and I-3470, respectively. Probio'Stick[®] is packaged in 2-g sachets as a lyophilized powder and is microencapsulated to ensure improved resistance to stomach acid, allowing the bacteria to reach the GI tract. The placebo will contain the same carrier material that is similar in size, taste, and shape to the probiotic formulation. Both the Probio'Stick[®] and the placebo are to be consumed orally once daily with the same meal each day. Participants will be instructed to consume half of the powder, swallow, and then consume the rest. Compliance will be monitored via questionnaire at each visit. In addition, participants will be asked to return any unused IP at each clinic visit.

Randomization and Blinding

Randomization will be accomplished by an unblinded research assistant not otherwise involved in the study. A simple randomization table will be generated using the website randomizer.org. All participants, investigators, LHS representatives, and coordinators performing assessments will be kept blinded as to the IP by using unique study identifications rather than any identifying information until week 8 of the study (phase I). For the remaining 8 weeks, the study will be single blinded only to participants. The study pharmacist will be unblinded for the duration of the study and will only perform study IP administration and IP accountability.

Table 3. Exclusion criteria

- 1 Current use of any antidepressant drug
- 2 Three or more previous episodes of depression
- 3 Failure to respond to another treatment in the current episode
- 4 Use of any antibiotic in the past 4 weeks (may be eligible to participate after a 1-month washout period)
Should a participant require an antibiotic during the 16-week study period, they may continue the study but must take the probiotic 4–6 h before or after intake of the antibiotic.
- 5 Use of any sleep medication in the past 4 weeks (may be eligible to participate after a 1-month washout period)
- 6 Milk, yeast, or soy allergy
- 7 History of alcohol or substance dependence in the past 6 months
- 8 Use of a probiotic product in the past 2 weeks (may be eligible to participate after a 2-week washout period) and during the trial
- 9 Use of any type of laxative
- 10 Use of non-steroidal anti-inflammatory drugs during the trial
- 11 Consumption of products fortified in probiotics 2 weeks before and during the trial
- 12 High risk of suicide (score ≥ 4 on item 10 of MADRS)
- 13 Psychotic symptoms determined by the MINI
- 14 Bipolar disorder determined by the MINI
- 15 Women who are pregnant, breastfeeding, or planning to become pregnant during the course of the trial (determined by a pregnancy test performed at the screening visit and confirmation of the use of appropriate contraception)
- 16 Immunodeficiency (immunocompromised and -suppressed participants; e.g., participants with AIDS, lymphoma, chemotherapy, or on long-term corticosteroid treatment, or allograft recipients)
- 17 Unstable or severe medical conditions (e.g., cancer, cardiovascular, renal, lung, psychiatric, or bleeding disorders, or diabetes, etc.)
- 18 The use of natural health products (e.g., St. John's wort, melatonin, or passion flower, etc.) that affect depression or sleep is not allowed in the trial
- 19 Electroconvulsive therapy in the year prior to participation in the study
- 20 Taking medication or other non-permitted treatment that cannot be safely discontinued

Outcome Measures

Clinical Measures

Clinical measures will be assessing changes in symptoms of depression and controlling for diet and GI symptoms. The primary outcome measure will be overall depressive symptomatology measured by the reduction in the total MADRS score. This will be measured every 2 weeks throughout the study. Additional outcome measures will include depressive symptoms measured with the QIDS-SR16, symptom severity and improvement using

the Clinical Global Impression Scale (CGI-S and CGI-I) [38], anxiety measured using the GAD-7 and the STAI, anhedonia measured using the SHAPS and Dimensional Anhedonia Rating Scale (DARS) [39], behavioral inhibition and activation using the Behavioral Inhibition System (BIS) and Behavioral Activation System (BAS) [40], personality using the NEO Five-Factor Inventory (NEO-FFI) [41], physical activity using the International Physical Activity Questionnaire (IPAQ) [42], quality of life using the Quality of Life, Enjoyment, and Satisfaction Questionnaire (Q-LES-Q) [43], and World Health Organization Quality of Life Assessment (WHOQoL-BREF) [44], functional disability and impairment measured using the Sheehan Disability Scale (SDS) [45], occupational functioning assessed using the Lam Employment and Satisfaction Questionnaire (LEAPS) [46], sleep quality measured subjectively using the PSQI, and cognition assessed using CNS Vital Signs (CNSVS) [47], which includes tests for verbal and visual memory and shifting attention. Dietary habits will be assessed using the Canadian Diet History Questionnaire (C-DHQ-II) [48], and side effects will be monitored using the Toronto Side Effects Scale (TSES), a 32-item scale that measures incidence, frequency, and severity of side effects associated with antidepressants [49]. Two environmental assessments will also be conducted over the phone: the Childhood Experience of Care and Abuse (CECA) interview and the Life Events and Difficulties Schedule (LEDS) interview to evaluate early life experiences and stressful events. Data collection time points vary for each measure (see Table 4 for details).

Polysomnography

In addition to measuring sleep quality subjectively, objective sleep architecture will be assessed using a portable Pursuit Sleep System and MediPalm from Breabon Corporation PSG machine. Sleep quality aspects of interest include percentage of time spent in each sleep stage, percentage of time spent in REM sleep, REM latency, and sleep continuity. PSG data will be collected at baseline, week 4, and week 8.

Molecular Analysis

Participants will provide blood, urine, and stool samples at baseline, week 2, week 8, and week 16. Venous blood will be collected in 6- and 10-mL ethylenediaminetetraacetic acid (EDTA) blood tubes and 8.5-mL PAXGene deoxyribonucleic acid (DNA) tubes; 10-mL EDTA tubes will be centrifuged in a refrigerated centrifuge to obtain plasma which will be divided into 8.2-mL cryovials and stored at -80°C . Venous whole blood from the one 6-mL EDTA tube will be divided into 3.2-mL cryovials and stored at -80°C for DNA for other molecular outcomes. Venous blood from the remaining 6-mL EDTA tubes will be passed through LeukoLOCK filters and stored at -80°C for RNA for mRNA and miRNA sequencing. Venous blood collected in the PAXGene DNA tubes will be stored at -80°C for DNA for methylation analysis. Urine samples will be collected in sterile urine containers and centrifuged in a refrigerated centrifuge. Urine supernatant will be divided into 2-mL cryovials and stored at -80°C . Stool samples will be collected in 90-mL sterile specimen containers and stored at -80°C . All biological samples will be banked for later use.

Neuroimaging

Participants will undergo magnetic resonance imaging (MRI) at two time points throughout the study to reflect changes in brain

Table 4. Study visit schedule

Procedure	SV	Week 0	Week 2	Week 4	Week 6	Week 8	Week 10	Week 12	Week 14	Week 16
<i>Clinical</i>										
ICF and review of criteria and sign-off	X									
MINI	X									
Demographics	X									
Antidepressant history form	X									
Physical examination	X									
NINDS medical history	X									
Pregnancy test (females)	X									
Randomization		X								
MADRS	X	X	X	X	X (phone)	X	X	X	X (phone)	X
CGI		X	X	X		X	X	X		X
TSES			X	X			X	X		X
CECA		*No set time point								
LEDS		*No set time point								
SHAPS		X		X		X				X
DARS		X		X		X				X
QIDS-SR16		X	X	X		X	X	X		X
GAD-7		X		X		X				X
STAI		X		X		X				X
BIS/BAS		X		X		X				X
NEO-FFI		X								
IPAQ		X				X				X
Q-LES-Q		X				X				X
WHOQoL-BREF		X				X				X
LEAPS		X				X				X
SDS		X				X				X
C-DHQ-II		X								X
PSQI		X		X		X				X
CNSVS		X				X				X
<i>Polysomnography</i>										
PSG recording		X		X		X				
<i>Neuroimaging</i>										
MRI, fMRI, DTI		X				X				
<i>Molecular</i>										
Blood samples		X	X			X				X
Fecal samples		X	X			X				X
Urine samples		X	X			X				X

Week 0: baseline; weeks 2–16: ± 3 days. ICF, informed consent form; SV, screening visit.

structure and function associated with IP consumption. The 3.0-T scan will consist of a whole-brain T1-weighted anatomical scan, a whole-brain T2-weighted blood oxygenation level-dependent (BOLD) echoplanar imaging (EPI) series during the awake resting state, a BOLD EPI series during a hedonic function task, and a BOLD EPI series during an affective go/no-go task. The hedonic task involves receiving feedback and rewards when correctly selecting responses in series of visual stimuli, and the affective task involves being presented with a series of visual stimuli containing emotional content and deciding whether to press a button depend-

ing on the nature of the stimulus. Scans will take place at baseline and week 8.

Mobile Health

In a subset of randomly selected participants, mobile health technologies (M-Health) will be used to examine changes in behavioral measures associated with IP consumption. Participants will load a designated study App designed to collect moment-to-moment information on their personal mobile device. This App will run continuously in the background of participants' mobile

Fig. 1. Summary of the protocol design.

phones to passively collect behavioral, context-sensing data on physical activity, geographical mobility, sleep parameters, and technology use – i.e., potential digital biomarkers of treatment response. Participants will also occasionally be prompted to actively complete 3 questionnaires: the Photographic Affect Meter (PAM) [50], Patient Health Questionnaire (PHQ-8) [51], and a brief sleep questionnaire.

Recruitment

Participants will be recruited from the community via both paper and web-based advertising. Paper advertisements will be posted on university and college campuses, community bulletin boards, and doctor's offices around the city of Kingston, ON, Canada. Online advertisements will be posted to Facebook and Kijiji. We expect recruitment to last for 24 months. Recruitment rate will be determined by availability of participants (based on inclusion/exclusion criteria) as well as resource limitations.

Prohibited Foods/Medication

Participants will be asked to maintain their regular diet during the study. Participants will be asked to discontinue any use of probiotic supplements and foods containing added probiotics or prebiotics such as yogurts or drinks with added probiotic cultures, snack bars, and cereals that contain probiotics, and foods containing any of the following 4 ingredients: inulin, chicory root fiber (or extract), oligofructose, or fructooligosaccharide. Medications not allowed during the study are listed in the exclusion criteria.

Analyses

Sample Size

Sample size was calculated based on the standard deviation of population MADRS scores as well as MADRS scores of depressed patients consuming Probio'Stick[®] from our pilot data and resulted in a sample size of 43 participants per arm for a total of 96 participants. Accounting for 25% attrition, we conclude that recruitment

of 54 participants per arm for a total of 108 participants will yield a sufficient sample size for 80% power at 0.05 level of significance (Fig. 1).

Statistical Analyses

Changes in clinical measures from baseline to weeks 8 and 16 will be analyzed with ANOVA using the statistical program Statistical Package for the Social Sciences (SPSS) with a significance level of $\alpha = 0.05$. Raw data from PSG will be analyzed by a trained sleep study technician and a quantitative report sent to research staff. Changes from baseline to weeks 4 and 8 will also be analyzed with ANOVA using SPSS.

Neuroimaging Analysis

Neuroimaging data will undergo manual quality control by a trained rater, followed by appropriate preprocessing for analysis. Analysis may include identifying structural changes and/or functional changes within the default mode network. Final analysis, however, will follow results from other ongoing work within the CAN-BIND network.

Biological Sample Analyses

Molecular analysis of plasma samples will include inflammatory markers such as the cytokines interleukin (IL)-1, IL-6, IL-17, tumor necrosis factor (TNF), and C-reactive protein (CRP), as well as the bacterial product LPS. LPS will be measured using a TLR4-reporter bioassay [52]. The cytosolic protein intestinal fatty acid binding protein (I-FABP) will be measured by enzyme-linked immunosorbent assay (ELISA). Gut microbiota composition will be determined by 16S rRNA gene sequencing [53]. Additional transcriptomic and genomic analysis of RNA and DNA will be determined based on results from other ongoing work and may take the form of a targeted validation or a discovery screen in these new samples. Metabolomics, both targeted and untargeted, will be conducted using participants' urine and plasma samples.

Approvals and Registrations

Ethics approval was obtained from the Queen's University Health Sciences and Affiliated Teaching Hospitals Research Ethics Board (protocol No. 6020656) on July 31, 2017. Authorization from Health Canada was obtained for use of a probiotic supplement in the form of Probio'Stick[®], a natural health product registered with the Natural and Non-Prescription Health Products Directorate of Health Canada. The trial was registered with ClinicalTrials.gov on September 1, 2017 (NCT03277586) and is being funded by LHS and the Ontario Brain Institute.

Results

Participant recruitment began in April 2018. We expect results from this trial to replicate and expand on results from the pilot study by showing improvements in these symptoms. We expect to see these outcomes mediated by decreases in inflammatory markers. Further, with the addition of neuroimaging and additional molecular measures, we hope to identify biological markers that will help predict responsiveness to probiotic supplementation in MDD.

Discussion

Despite advances in depression research, it remains a challenging disorder to treat. This is in part due to the failure of current pharmacological treatments [54]. Thus, identifying novel treatments and treatment targets is key to advances in depression research. To the best of our knowledge, this will be the first blinded, randomized, placebo-controlled trial assessing the effects of a probiotic supplement on symptoms of depression in MDD patients controlling for severity, chronicity, and treatment resistance of the disorder. We hope to provide evidence of efficacy for a new option for alleviating symptoms of depression. Inside the CAN-BIND network, we also hope to elucidate the neuropathology of this disease by isolating biomarkers that predict responsiveness to the probiotic.

Effective first-line antidepressant medications are currently limited. Many patients try 2–3 different formulations or combinations before achieving symptom relief, delaying the onset of possible recovery. Even then, up to 60% of patients discontinue their antidepressant use within 3 months [55]. One of the most common reasons for this is side effects, which can include nausea, vomiting, headache, agitation or sedation, sexual dysfunction, and weight gain [49, 56]. In 2013, a study by Bet et al. [56] showed that over two-thirds of patients experience up to

3 side effects that often persist throughout long-term antidepressant use. Moreover, many individuals do not seek treatment for their depression at all due to the stigma associated with mental illness and antidepressant medication, preventing recovery and perpetuating the disease cycle. Probiotic supplementation is considered low risk and may be a favorable alternative to classical antidepressants due to the absence of side effects or adverse reactions noted in our pilot research. Furthermore, probiotic supplementation does not carry with it the stigma that antidepressant medications do, an issue that can be a barrier to seeking treatment. Finally, probiotic supplementation may even lead to improvements in overall general health [57].

Possible limitations of this trial include issues with participant recruitment and attrition, and failure to comply with the IP regimen. Finding individuals who are depressed and not currently taking any medication to participate in research can be difficult, and adherence to medication is typically lower in patients who present with depression compared with those patients who do not [58]. These issues will be minimized by directing a substantial amount of time and resources to advertising and recruitment, and having the study explained to the participant in detail at the screening visit. The IP will be clearly labelled and packaged for easy use, and participants will be followed up biweekly and monitored for adherence via questionnaire at each follow-up. Other limitations include selection bias in recruitment and the unblinding of study personnel in phase II, which carries the risk of confirmation bias.

In summary, there is a critical need for blinded, randomized, placebo-controlled trials in clinical populations using novel antidepressant therapies. This study will contribute to the growing body of nutritional psychiatry research by assessing the efficacy of dietary probiotics on depressive illnesses and attempt to disentangle the underlying mechanisms of this relationship.

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Statement of Ethics

The study protocol has been approved by the Queen's University Health Sciences and Affiliated Teaching Hospitals Research Ethics Board. All subjects gave their written informed consent.

Disclosure Statement

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Author Contributions

C.J.K.W. and R.M. developed the protocol for the study. All authors provided their individual expertise and read and approved the final manuscript.

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