Table of Contents

Context **2**

Purpose/Objective **2**

Hypothesis **2**

Study Design/Setting/Participants **2**

Inclusion/exclusion, consent and randomization 2,3

Sample collection 3

Stool sampling and study schedule 3

Time requirements 4

Population group/ inclusion criteria 4

Outcomes and statistics **5**

Treatment and control product information **5**

Recruitment and Compensation **5**

Data collection, privacy, and confidentiality **5**

Data anonymization, privacy and confidentiality 6

Stool samples/Microbiome 6

Health coach check ins 6

Blood panel 6

Risk Assessment 7

Study Interventions and Measures 7

Risk-Benefit 7

Funding and Declared interests 7

References 8

Appendix 8

1 Participating Clinics 8

2 Manufacturers 8

3 Study Questionnaire 9

4 Recruitment Info Sheet 10

5 Labs 12

Title: Enhanced probiotic pilot study
Date: 5/1/18

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Address: 2455 Dunstan Rd. Apt #581 Houston, TX 77005
Principal Investigator: Dr. Eric McLaughlin

Context

Antibiotics are a powerful tool to combat microbial infections—-but as collateral damage, they can destroy the gut microbiome. Therefore, it has become common practice to take a probiotic supplement to offset side effects that are a result of antibiotic use, albeit with moderate success (Johnston, Goldenberg, Parking, 2016, McFarland 2015). Fecal microbiota transplants have been used in extreme cases to restore a healthy and diverse microbiota in patients with severely destroyed microbiomes (Kakihana et al. 2016). Yet, many of the strains in a fecal transplant are obligate anaerobes, and therefore not readily available for preventative treatments. Strategies that are more effective in restoring the microbiome after antibiotic assault on the gut flora are missing.

Purpose/Objective

To see if an enhanced probiotic can help maintain and restore balance in the body and the gut microbiome more quickly and effectively than a probiotic alone.

Hypothesis

If we provide [redacted] compounds from microbial activity that simulate and stabilize a healthy gut ecosystem, we can support the gut's natural ability to rebuild a normal microbiome population after antibiotic assault. A faster rate of return to a healthy microbiome is a marker of overall health and may help reduce negative side effects of antibiotic use such as yeast infections, acne, diarrhea, bad mood, and inflammation.

Study Design/Setting/Participants

Inclusion/exclusion

The study will take place in clinics run by or partnered with our principal investigator (contact information in the appendix). Our subjects will go over the inclusion/exclusion criteria (see pg 4) with the investigator or sub-investigators.

Consent

If the participants qualify they will be informed about the purpose of the trial, and directed to an empty room where the study sponsor is located. An onsite nurse, trained in human research, will review the consent form with the potential participants and answer any questions about the study and its potential risks. The sponsor will leave the room during the entire consent process, but will be available for questions, if needed. The participant will be offered time and privacy to review the consent form (the nurse will leave the room). The participant may also take the consent form home to review. If they choose to participate they must sign the consent form. Consent forms will also be signed by the principal investigator or sub investigator.

Randomization
If a patient chooses to participate, a staff member of the clinic or a Postbiotics Plus representative will choose an unmarked box (the study kit) containing stool sample materials, and either control (C) or treatment (T) materials. Boxes have been randomly shuffled after assembly, and contain barcodes that will allow the sponsor to identify the participant's treatment arm after the participant hands over their samples. The staff member or the Postbiotics Plus representative will not know the treatment arm during the handover of the study kit.

Sample collection
We will ask participants to collect five stool samples during and after their course of antibiotics (see study schedule). For our primary outcome—-protection from microbiota diversity loss—-we require a sample at the end of the antibiotic course. To maximize compliance and protect against complications to produce a sample on the very last day, we ask participants for a sample 2 days before the scheduled end of their antibiotic course (with the option to submit a sample from the last day in case they were unable to produce a sample).

Stool sampling and study schedule
(as provided to the participants)

	Day 1 antibiotics (abx)	2 days **before finishing** abx	Day 1 after finishing abx	Day 2 after finishing abx	Day 3 after finishing abx	Day 10 after finishing abx
Stool Sample		X	X	X	X	X
Blood Sample						X
Check in	X	X	X		X	X

Important: If you are unable to produce a sample 2 days before finishing the antibiotics (abx), please try on the final day of your antibiotic course. Should you be unable to produce a sample on any other day, please try on the following day and record the correct day in your log.

Norgen Biotek stool collection and preservation kits for microbiome sampling and the enhanced probiotic or control will be provided to the participants when they enroll.

Instructions provided to patients for stool collection:.

- Put on the gloves provided
- Wrap the paper collection kit around toilet as shown
- Complete a bowel movement
- Take the tube, unscrew the cap and scoop out a small sample of stool from three different sections of the stool
- Place the sample collected in the tube and screw the cap back on tightly
- Place the time/date of your sample on the form attached and place the barcode sticker on the form and the matching barcode on the appropriate sample. Keep the barcodes in numerical order. Also record this information online via your Fruitstreet.com platform.

- Place tube in the collection bag provided and then store the tube inside the mailer provided
- Break off paper collection and flush stool as shown
- Store the mailer with your samples in a cool, dry place, approx 70 degrees until you have collected all 5 samples. The samples MUST stay COOL.
- When you have collected all 5 samples in the mailer provided, place the samples and the collection sheet with your barcodes and time/date recorded in the mailer to bring with you to a participating clinic.

Samples are stabilized and will be stored in biohazard bags in designated area at each site until collected at the end of the study.

Check ins
We will check in with every participant the day before each stool sample and also ask them to report any adverse side effects. If any side effects occur the principal investigator will be notified immediately and a report will be written and handed into the IRB.

Blood Work & Sample Return
Participants will be asked to visit participating clinics at the end of the study, post treatment, to have their blood drawn to measure inflammation markers.

Time requirements
The total amount of time required of the participant will be approx 4 hours over the course of 20 days. Duration may vary depending on the course of antibiotics.

Population group/ inclusion criteria

Participants will be provided by doctors and clinics in the area. We will enroll up to 50 individuals in order to achieve our goal of 25 evaluable participants.

- >25 participants, up to 50
    - 13 T: Treatment, combination of probiotic [redacted] and fermented herbs
    - 12 C: Control, standard probiotic [redacted]
- Healthy adults with a BMI (18-28)
- Patients are prescribed antibiotics for a course of 5 days or longer
- Patients have not had another course of antibiotics in the past 6 months.
- Patients are willing to cease taking any other supplements or probiotics.
- Patients will be assessed by their doctor for the following exclusion criteria.
    - Exclude patients suffering from gut related illness (regular and severe constipation, diarrhea, inflammatory bowel disorder or Crohn's disease).
    - Exclude patients who usually experience severe diarrhea (e.g. for more than 3 days) following antibiotic treatment.
    - Exclude patients who have a compromised immune system, are taking any immune modulators or have an autoimmune disorder.
    - Exclude patients who are diabetic or take any blood pressure medication.
    - Exclude patients who have any known allergies to the herbs or probiotics in the the formula.

- Exclude patients who are pregnant or nursing.

Outcomes and statistics

First, we will compare the diversity at the end of the antibiotic course between the two treatment arms to assess if patients who received the combined product (T) have a higher microbial diversity at the end of the antibiotic treatment than those who received a standard probiotic (C). We will then use time series analysis methods to assess recovery of the microbiome. We will use a hierarchical model with random effects per patient to assess if patients of treatment arm T have higher diversity over the period of recovery.

We will measure inflammation markers, C-reactive protein, to elucidate the utility of monitoring inflammation as secondary endpoint for future studies.

Treatment and control product information

Every participant gets a treatment box, with two white pill containers. Every box contains one bottle filled with probiotics off the shelf and the second bottle will be filled with orange capsules of either a booster, our fermented herbal product, or a placebo, corn starch. The boxes are coded with bar codes to identify if the patient received the booster or the placebo in addition to their probiotics. Neither the sponsor nor the participant will know which they are given. The participant will take both products products separately, a probiotic and the booster or placebo. The booster is the investigational product. The probiotics are in white capsules in a white pill container. The booster or placebo are in orange capsules in another white container identified with a sticker. The booster and the placebo are packaged identically. [redacted] was chosen as the probiotic as it is commonly used with physicians and has been tested throughly on its own merit.

Participants are instructed to take two white capsules, the probiotic, with 1 orange capsule every day. [redacted] label reads that up to 16 capsules can be taken as a serving. We intentionally choose a low dosage.

The [redacted] will be purchased by the sponsor, but approved by physician. The sponsor will also provide the herbal booster or control.

Treatment Product Box (T): The participant will be given a box that includes one bottle of [redacted] probiotic blend, in white capsules and one bottle containing 650 mg orange capsules of fermented blend [redacted] Both are produced by certified manufacturers who currently sell supplements in the US. See appendix for manufacturer contact information.

Control Treatment Box(C): The participant will be given a box that includes one bottle of [redacted] robiotic blend, in white capsules and one bottle containing 650 mg orange capsules containing microcrystalline cellulose, the control.

Both treatment arms will appear identical and the participant will not know if they are taking the combined product or the control. Both, C and T are to be taken daily during the course of antibiotics, and for 10 more days thereafter. Sufficient supplies of either C or T will be provided.

Recruitment and Compensation
Participants will be recruited by physicians. If they fit the criteria for the study they will offered the opportunity to speak with the nurse trained in human research and the study sponsor. Participants will be compensated $15 on an amazon gift card per sample collected at the end of the study. If a participant does not complete the study they will no be penalized, and will be rewarded for the samples they do collect.

Data collection, privacy, and confidentiality
All participants must sign an informed consent form to participate. Their data will be de-identified and stored for future research.

Data anonymization, privacy and confidentiality
Submitted samples will be assigned a unique anonymous identifier. Keys to the identifiers will be stored securely on a separate hard drive from the data and be accessible only to the sponsor. All other persons who require access to the data (including Diversigen who perform sequencing) will be given anonymized data only. At the end of the study, and once microbiome data sheets have been mailed, identifier keys will be destroyed and only anonymized data will be retained.

Initial processing of sequencing data will be performed by Diversigen using their standard data processing pipeline. Diversigen will produce data tables containing information of relative operational taxonomic unit data (OTU tables). These will then be used for further analysis by Postbiotics Plus.

Stool samples/Microbiome

All sequencing of anonymized samples will be conducted by our partners at Diversigen (https://diversigen.com/)

- Metagenomic data will be analyzed using standard bioinformatic tools
- Diversigen will provide OTU tables after all samples have been sequenced.
- Alpha-diversity and other community metrics will be calculated using scikit-bio (http://scikit-bio.org/).

Blood panel
High sensitivity C-reactive protein will be collected at participating clinics and labs post treatment to elucidate the utility of monitoring inflammation as an endpoint for future studies.

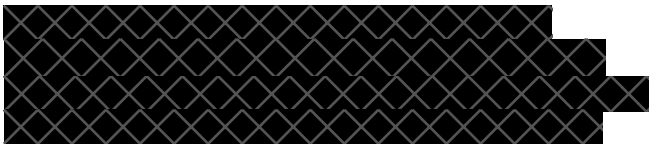
Blood panel time points

- Right after combined product (T) or the control (C) course is completed, i.e. 10 days after antibiotic course finished.

Risk Assessment

All ingredients of the fermented herbs are GRAS status and currently sold in the US. Minimal risk of allergy or adverse reaction. Fermented herbs and probiotics have the potential for some to cause bloating or gas, an upset stomach or loose stools.

The supplements used in this study have not been FDA approved. The individual ingredients have not been shown to interfere with the antibiotics prescribed, but their combination has not been tested, therefore some unknowns exist.



Although we will do our best to protect participants study information, there is still a very small risk of loss of privacy.

Study Interventions and Measures

Our primary investigators will oversee all participants by receiving updates from every check in call (prior to each sample time) to monitor any adverse symptoms or reactions. Any adverse reactions must be reported and recorded by the sponsor and reported to the principal investigator. Check-ins will occur according to the study schedule. If any should occur, the participant will stop product use immediately and the investigator will be notified. They will either be referred back to their primary care doctor or provided support to find relevant medical attention. Participants will also be given a 24 hour number to contact the study sponsor and all participating clinics provide 24 hour emergency care.

Risk-Benefit

Our study poses minimal risk. Supporting the recovery of the microbiome can be beneficial for overall health and help remedy discomfort, reduce diarrhea, and potentially reduce frequency of other antibiotic related side effects or complications.

Funding and Declared interests

The principal investigator will fund the pilot study. However, he will not participate in the data analysis. The sponsor was involved in the study design and will provide all study materials to the participant, free of charge. The sponsor will oversee all study activities and coordination.

References

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Appendix

1. Participating Clinics:

Baymont Emergency Room, LLC
Assumed Name:Patients Emergency Room
10133 Interstate 10 East
Baytown, TX 77523
EIN: 46-4901030

Elite Care Emergency Center
Address: 2500 Rice Boulevard, Houston, TX 77005
Phone: (713) 527-4400

2: Manufacturers
Herbal ferment:
AGM Foods Pty Ltd
The Home of Grainfields Australia
Manufacturer and Wholesaler of Probiotic Superfoods
agmfoods.com

VSL#3 *probiotic:*
Viale Shakespeare 47
00144 Rome (Italy)
Vsl3pharma.com

3: *Recruitment Info Sheets*

Dear Participant,

Thank you for your interest in our study.

The purpose of this study is to improve our understanding of how an enhanced probiotic can speed up recovery of the gut after antibiotic use and influence gut health, inflammation and overall health.

Antibiotics are a powerful tool to combat infections—-but they can also negatively affect the gut. While the gut can recover naturally, sometimes this can take a while, potentially leaving room for unwanted side effects.

STEP 1: Determine if you fit the Inclusion Criteria to participate.

- You have been prescribed an ntibiotic.
- You are generally healthy adult.
- You are willing to stop taking other supplements or probiotics.
- You have not had another round of antibiotics in the last six months.
- You are not regularly suffering from gut related illness (regular and severe constipation, diarrhea, inflammatory bowel disorder or Crohn's disease).
- You are not usually experiencing severe diarrhea (e.g. for more than 3 days) following antibiotic treatment.
- You do not have a compromised immune system, suffer from an autoimmune disorder or take an immune modulator.
- You are not diabetic or on any blood pressure medication.
- You do not have any known allergies to probiotics, ashwagandha, astragalus, red lentils or elderberry.
- You are not pregnant or nursing.

STEP 2: Decide if you are willing to do what will be required.

- We will be asked you to collect stool samples! But we will try and make it as easy and simple as possible.
- You must take your probiotic every day
- You will check in with a health coach during the study
- You will be asked to return your stool samples and have your blood drawn at the end of the study

STEP 3: Contact for more information and any questions: cell: 832.238.1781, 24-hour availability, or email: research@postbioticsplus.com

You may also visit:
Baymont Emergency Room, LLC
Assumed Name:Patients Emergency Room
10133 Interstate 10 East
Baytown, TX 77523

Thank you!

Postbiotics Plus Team