Bismuth Subsalicylate's Role in the Prevention of Travelers' Diarrhea and Impact on Acquisition of Gut Antimicrobial Resistance Genes

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Project Overview

Protocol Summary

The purpose of this study is to determine if the use of prophylactic bismuth subsalicylate (BSS) has an effect on the acquisition of travelers' diarrhea (TD) or antimicrobial resistance (AMR) genes in fecal samples among international travelers who departed from the United States to South East Asia, South Central Asia, or Africa. Our hypotheses will be tested using a double-blinded, placebo controlled randomized clinical trial with participants from a pre-travel health clinic in the United States (see Study Design and Location). To be eligible to participate, travelers must be at least 18 years and no older than 70 years of age, travel internationally to South East Asia, South Central Asia, North Africa, or Sub-Saharan Africa for 7 to 21 days, be non-pregnant and non-nursing, and be willing to participate. We expect, based upon prior studies (see Current State of Knowledge), to see a decreased incidence of TD among participants in the intervention group. We also believe, based upon known properties and actions of BSS, that there will be reduced acquisition of gut AMR genes in the intervention group. We hope findings from this study will be used to help international travelers prevent TD and changes to their intestinal resistome, thereby decreasing the spread of AMR genes across international borders.

Accelerated Protocol Review

None needed.

Funding Mechanism

This will be funded under the 28th Amendment to a Cooperative Research and Development Agreement (CRADA) between CDC and P&G (expiration: June 30, 2018). A new Amendment will be instated to allow continuation beyond the expiration date. The New York Center for Travel and Tropical Medicine clinic will be the lead of the project. Funds will also be provided directly to the New York Center for Travel and Tropical Medicine for clinic activities.

Investigators/Collaborators

Principal Investigators:

The New York Center for Travel and Tropical Medicine clinic director Bradley A. Connor, MD will serve as a co-Principal Investigator(s) (co-PI) with the CDC's Kristina M. Angelo, DO, MPH&TM (co-PI).

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Dr. Connor's team at The New York Center for Travel and Tropical Medicine will lead this study. They will perform study subject consent, enrollment, double-blinded assignment of study subjects to intervention and placebo groups, data (both questionnaires and biologic [stool] sample) collection, and response to traveler questions during travel as required. They will also manage the IRB submission.

Dr. Angelo's team in the Travelers' Health Branch at CDC will assist with the study design and protocol development, conduct the blinding of placebo and intervention medications, perform data analysis of both questionnaire and biologic (stool) specimen data including data interpretation (with coded data), and provide epidemiologic and travelers' health subject matter expertise and support to Dr. Connor's team.

Any publications using this data will require with the New York Center for Travel and Tropical Medicine clinic's involvement and oversight. CDC will not interact with any research subjects or have access to or handle any personally identifiable information, with the exception of a limited number of information technology personnel responsible for REDCap software maintenance, who are not participating in the study or protocol. CDC will not retain or claim ownership of the resulting data. CDC will be providing a secure data management platform (REDCap) to the New York Center for Travel and Tropical Medicine; CDC will have access to de-identified data only with the approval of Dr. Connor and his team. A nondisclosure agreement with The New York Center for Travel and Tropical Medicine will be signed; the clinic will not provide any PII (or the key to the coded PII) to any CDC staff.

The Clinical and Environmental Microbiology Branch at CDC will assist with study design, receive all biologic (stool) specimens for testing (coded; no PII will be sent with the specimens), and perform all laboratory analyses as indicated per the protocol. They will also store biologic (stool) specimens for further analysis.

The Enteric Diseases Epidemiology Branch at CDC will assist with study design and protocol development, data interpretation, and will provide epidemiological, statistical, and enteric diseases subject matter expertise and support.

P&G will not be involved with execution of the protocol. They will provide all intervention and placebo group medications and funding for the study.

Conflicts of Interest

P&G states that their purpose is to improve the lives of the world's consumers, and are interested in knowing whether their products and services are effective in this capacity. Because it is a publicly-traded private, for-profit corporation, P&G must develop products that consumers find useful enough to purchase regularly. This study will help the company understand more fully how its products further their mission to improve lives, may help the company develop new marketing strategies for their products, or modify existing formulations of their products to be more effective at preventing intestinal colonization with potentially harmful microorganisms.

Introduction

Current State of Knowledge

Travelers' diarrhea (TD) is the most common travel-related illness reported by international travelers, affecting between 30–70% of all travelers (1). The incidence of TD varies by travel destination, duration of travel, season, travel activities, and personal traveler attributes. Some areas of the world are considered high-risk, including Asia and Africa, but also the Middle East, Mexico, and Central and South America (1). Previous placebo-controlled clinical trials with the prophylactic use of bismuth subsalicylate (BSS) demonstrated a significant reduction in the incidence of TD among those receiving the drug. A recent unpublished meta-analysis on the subject of BSS prophylaxis found that persons who took BSS while traveling internationally before the onset of gastrointestinal symptoms had a 3.5 times greater odds of remaining free of TD in comparison to those who received placebo (2). BSS is available in both liquid and tablet formulations; both formulations reduced TD at doses of either 60mL four times daily (4.2g/d) for the liquid formulation (3) or 2 tablets, four times daily (2.1 g/day) for a maximum of 3 weeks (3, 4, 5). BSS at 2.1 g/day protected 41–65% of participants (3, 5), while the liquid formulation protected 62% of participants (3). Studies suggest the lowest minimum dose for protection may be 1g daily taken in 2 doses, which provided 35% protection in one analysis (5) and 40% protection in others (3, 4). BSS had minimal side effects, but these may include nausea,

constipation, tinnitus, and blackening of the tongue and stool (5, 6). BSS may cause encephalopathy if taken in high doses for prolonged periods, but this is exceedingly rare and above the recommended dose (7, 8).

The active component of BSS with antimicrobial effects is bismuth, which is poorly absorbed and mostly remains in the GI tract while salicylate is systemically absorbed in the stomach (9). The mechanism of action of BSS in the prevention of TD is thought to be due to bismuth's intraluminal antimicrobial properties and a reduction in the number of bacteria (4, 6, 9, 10). In-vitro studies suggest that bismuth's action is bactericidal (6, 11), even at various levels of intestinal pH and against a wide range of organisms (11). The precise mechanisms of action include the binding of ingested bacteria and subsequent marking of these organisms to be killed by host defenses, rendering the bacteria unable to attach to receptor sites that are important for colonization and infection (11), degrading the bacterial cell wall, inhibiting ATP synthesis, and inhibiting the function of the plasma membrane (9). This inhibition may occur in as little as 0.5–24 hours (9). BSS may also inactivate bacterial toxins before attachment to the intestinal mucosa (12). Viruses may be inhibited by BSS; it may prevent viral invasion and deter replication through unknown mechanisms (4).

There is a current domestic and international focus on preventing the acquisition of drug-resistant enteric bacteria by high-risk groups, including travelers (13). International travelers are at increased risk for acquiring gut organisms containing AMR genes (especially after travel to Asia), developing TD, taking antibiotics, and requiring hospitalization (14, 15, 16, 17). Although carriage of organisms containing gut AMR genes may be transient in most travelers (15), it can persist for ≥1 year in over 10% of travelers, increasing the risk of transmission to household members and others (18). Avoiding the use of antibiotics while abroad is one way to lessen acquisition of antibiotic-resistant organisms (19). BSS treatment of adult outpatients with acute diarrhea has been found to reduce antimicrobial medication use in a high prevalence area (20). It is currently unknown whether BSS affects the acquisition of gut AMR genes while traveling abroad; however, given BSS' antimicrobial properties, stability in acidic conditions, and blockage of receptor sites to prevent colonization, it is possible that prophylactic BSS may indirectly prevent acquisition of AMR genes in international travelers by preventing intestinal colonization of ingested microbes. More research must be done to address this uncertainty.

Justification for Study

International travel has expanded over the past 50 years. Worldwide annual tourist arrivals are expected to reach 1.8 billion by 2030 (21). Travel destinations continue to diversify, including increasing numbers of travelers visiting countries with emerging economies, such as Asia and Africa. These trends may place international travelers into closer contact with infectious pathogens, including those that cause TD and have AMR genes (21, 22). This may contribute to infectious disease spread across international borders. International travel, particularly to countries with emerging economies, is a well-documented risk factor for acquisition of gut AMR genes (23, 24). Also, acquisition of TD during travel has been associated with persistent infection or post-infectious irritable bowel syndrome (PI-IBS). PI-IBS is the onset of irritable bowel symptoms after an enteric infection, and may lead to a permanent change in bowel function (25).

Given the burden of TD and the possibility of acquiring AMR genes while abroad, there is ample justification for studying simple, cost-effective, and safe actions that could be taken by international travelers to reduce these risks.

Intended Potential Use of Study Findings

Healthcare providers and public health officials may use the findings from this study to enhance or change recommendations made to international travelers when offering pre-travel advice. Healthcare providers may advise travelers on improved best practices to decrease their risk of TD and acquisition of AMR genes. Public health officials may use information from this study to develop guidelines and advise on best practices to ensure that the impact of TD and drug-resistant organisms is minimized in international travelers. By minimizing acquisition of AMR genes in international travelers, we would also be helping protect the general, non-traveling public from exposure to imported antimicrobial resistant organisms. This study may also generate hypotheses that could help guide future study of possible mechanisms of action and uses of BSS in reducing the development of and treating TD.

The results will be made public through publication(s) within 18 months of the termination of data collection. This/these publication(s) will be in a peer-reviewed journal; however, the initial release may be in the form of an abstract and presentation at a scientific meeting (see Disseminating Information to the Public).

Study Design and Location(s)

This study will be conducted as a double-blinded, placebo-controlled randomized clinical trial. We propose two arms to test BSS against placebo:

1. BSS 4 tablets po bid (2.1 grams total of BSS)
2. Placebo 4 tablets po bid

Study participants will be recruited from The New York Center for Travel and Tropical Medicine Clinic in New York, New York, USA (see Procedures) (http://www.travelhealth.net/). Throughout the study period, the recruiting site will maintain adequate personnel trained in the study protocol and procedures (see Training for Study Personnel). Study personnel will be available to communicate with the co-PIs as necessary and to address issues with the study protocol and concerns of the study participants. This site will be able to consent and enroll participants (see Procedures for Implementing and Documenting Informed Consent), store and distribute the study drug and placebo, and collect and safely store questionnaire data (see Data Handling and Analysis) and biologic samples (stool) data (see Biologic [stool] Specimen Collection and Testing), in accordance with the study protocol.

Objectives

1. (Hereafter, "primary objective") Test the efficacy of BSS in the prevention of TD among international travelers.
2. (Hereafter, "secondary objective") Determine the impact BSS has in the acquisition of gut AMR genes among international travelers.

Hypotheses

The following hypotheses follow the objectives above:

1. NULL HYPOTHESIS: BSS does not prevent TD among international travelers.
    a. Does BSS prevent TD among international travelers?
    b. Does BSS reduce severity/frequency of TD among international travelers?
2. NULL HYPOTHESIS: BSS does not alter the acquisition of gut AMR genes among international travelers.
    a. Does BSS affect the acquisition of gut AMR genes among international travelers?

b. Does BSS affect the acquisition of gut AMR genes among international travelers that develop TD?
    i. Does BSS alter the acquisition of gut AMR genes if an international traveler with TD takes an antimicrobial while taking BSS?
c. Does BSS affect the acquisition of gut AMR genes among international travelers that do not develop TD?

General Approach

The approach of this study will be hypothesis-testing.

Procedures and Methods

Design

Statement of Purpose

The purpose of this study is to investigate the impact of BSS on the acquisition of TD and acquisition of gut AMR genes among international travelers.

Study Medications

The placebo, produced by P&G, will be comprised of the following components: microcrystal cellulose, calcium carbonate, D&C red No. 27 aluminum lake, flavor, magnesium stearate, mannitol, povidone, saccharin sodium, and talc. It will be formulated to have an appearance (size, shape, color, and packaging), texture, and taste similar to BSS.

Each bottle to be used in the study will be blinded by CDC personnel (but not the co-PI). Each bottle will include an internal study ID#, owner name, batch number, start date, and expiration date. The blinding label will additionally include a study ID for identification. Bottles of study medications will be sent to The New York Center for Travel and Tropical Medicine clinic for distribution to participants at their pre-travel consultation/enrollment visit.

Procedures

This study will be in the form of a double-blinded, placebo-controlled randomized clinical trial, conducted at The New York Center for Travel and Tropical Medicine clinic in

New York (see Description and Source of the Study Population). This clinic offers pre-travel specialty medical services to persons planning international travel and has the necessary resources, skills, and expertise to evaluate and treat travelers who acquire travel-related illness, and offer advice to travelers when questions or problems arise during their trips.

All travelers who are seen for a pre-travel consultation at The New York Center for Travel and Tropical Medicine will be administered the screening questionnaire by study personnel upon arrival to their consultation to determine their eligibility. Eligible travelers will be invited to participate in the study (see Participant Inclusion / Exclusion Criteria). All participants will be consented as per the protocol (see Procedures for Implementing and Documenting Informed Consent). Randomization of participants will be double-blinded and conducted via block randomization to ensure groups of equal sample sizes using a computer generated distribution. Study medications for both the intervention and placebo group will be provided by P&G, blinded by CDC (using a unique identifier), and distributed to participants at the clinic site during the pre-travel consultation/enrollment visit per the randomization mentioned above (see Study Medications). Study personnel at The New York Center for Travel and Tropical Medicine will not be aware of which medications are BSS or placebo. The code number of the medication (intervention or placebo) administered to a particular participant will be recorded. All code numbers and corresponding arms will be kept secret until the end of the trial.

Web-based questionnaires will be administered during and post-travel (see Study Instruments). Pre-travel data will be collected via telephone with study personnel. Pre-travel data will include information on the participant's medical history and travel plans, and post-travel data will include information on the TD symptoms, medication use, medical care received, and adverse events. During travel, the participant will complete a daily questionnaire that will include data on compliance with taking the study tablets, presence of symptoms of TD, onset of adverse reactions, any medical care received, and trip specifics. The during-travel questionnaire will be made available in both a web-based electronic form and a paper form in the event that the participant does not have reliable internet access. Any paper forms that were completed will be returned by mail

after they return from travel via a pre-paid envelope addressed to the study clinic that was given to the participant when enrolled.

To address the secondary objective, participants will also be asked to provide pre- and post-travel biologic (stool) samples for laboratory testing for the presence of gut AMR genes (see Laboratory instruments). Timing of the pre- and post-travel data and stool sample collections will be within 7 days before departure and 10 days after return from travel, respectively. Both biologic (stool) samples will be self-collected and submitted by mail to the clinic (the collection kit, and all necessary packaging and postage will be provided to the traveler). The participant will not be seen by a healthcare provider involved with the study post-travel unless they are ill and seek an evaluation.

As part of standard pre-travel consultation practices, if indicated, travelers will be prescribed anti-diarrheal medications and/or antibiotics for self-treatment of TD. These medications may include loperamide (Imodium), diphenoxylate and atropine (Lomotil), BSS (Pepto bismol), ciprofloxacin, azithromycin, or rifaximin. If these medications are taken while abroad, the travelers will be asked to note this on the during travel or post-travel questionnaires.

No difference in pre-travel advice, provision of anti-diarrheal medications, or during- or after-travel medical evaluation or advice will occur based on the traveler's assigned study arm.

Duration of Subject Involvement

The duration of each participant's involvement in the study is expected to be a maximum of 6 weeks. This will account for time to complete the pre-travel consultation/enrollment visit, 21 days (or less) of travel, and post-travel follow-up.

How the Study Design Addresses the Objectives and Hypotheses

A double-blinded, placebo-controlled randomized clinical trial is the best study design to address the study objectives and hypotheses. We aim to determine if there is a biologic benefit to an intervention (BSS administration), and a placebo-controlled methodology is required to determine if there is a statistically significant benefit of the intervention

regarding incidence of TD and acquisition of gut AMR genes. It is imperative to blind both healthcare providers and participants to ensure that biases, including selection and observer bias, are minimized.

Stakeholder Participation

The primary stakeholders for the project are international travelers, healthcare providers, and public health officials.

P&G cannot participate in the study procedures (i.e., data collection or analysis) to minimize the potential for any commercial influences or the appearance thereof. They will not be able to prevent the publication of negative results, nor influence the interpretation of the results. They will be responsible for providing both intervention medication and placebo.

CDC and P&G will operate under the terms of the CRADA regarding cooperative research, reports, financial and staffing obligations, patent applications, licensing, proprietary rights and publication, representations and warranties, disputes, and liability.

Cost Benefit/Prevention Effectiveness

A single 262mg tablet of BSS costs approximately $0.26 when purchased over-the-counter ($10.49 for 40 tablets). The cost of antibiotic treatment of TD or potential hospitalization from complications, or for diagnostic procedures or other treatment interventions, far surpasses the cost of BSS for possible TD prevention.

The cost benefit regarding the secondary objective is unable to be defined, however, the potential costs for spread of gut AMR genes from colonized international travelers are presumed to be large compared to the minimal cost of BSS prophylaxis. Further, there is no evidence currently that any microbial resistance to BSS exists, inducible or otherwise. Finally, any reductions in TD incidence among travelers that is related to prophylactic BSS use could result in a reduced need for and use of antibiotics by travelers, with a concomitant reduction in the selective pressures for antimicrobial resistance genes.

Description of Risks to the Participant

There are minimal risks to participants in this study. BSS has been marketed in the United States for over 100 years, and is widely available without a prescription. It has a good safety profile at the recommended dose and duration of use. We will not exceed these safe dosing ranges in this study. The maximum daily dose (4.2g per day) of BSS results in peak serum concentrations of salicylate considerably below toxicity levels. This dose has been documented to be safely used for up to 3–4 weeks (26). We will include only adult participants and exclude children <18 years of age or elderly adults 70 years of age or older, pregnant or breastfeeding women, participants with a known contraindication to BSS use, or anyone taking medications known to interact with BSS, including other salicylate-containing compounds. Participants will be asked to refrain from taking aspirin or aspirin-containing compounds. The duration of BSS administration in this study will be limited to a maximum of 21 days.

BSS lists possible side effects on its packaging (including, but not limited to, constipation and tinnitus) that will all be discussed with the participant before informed consent and enrollment.

Participants will be provided contact information for study personnel at The New York Center for Travel and Tropical Medicine in the event that they have questions regarding the study or the medications before, during, or after travel. They will be notified to contact study personnel at The New York Center for Travel and Tropical Medicine in the event that they experience any adverse events of BSS or symptoms of TD.

In addition to the study preparations (BSS or placebo tablets), participants may also be provided with an appropriate medication for TD self-treatment (at the discretion of the healthcare provider) to carry with them in the event they develop TD while abroad. If they experience an episode of TD, they will be instructed to continue their study medication. Participants will also be counseled on hydration, the indications for antibiotic use (*i.e.*, in the event of severe diarrhea, but not mild or moderate diarrhea), and when to seek healthcare.

Each participant's confidentiality will be rigorously maintained (see Procedures for Implementing and Documenting Informed Consent). If a breech occurs, the IRB will be notified immediately and standard procedures will be followed.

Description of Anticipated Benefits to the Participant

Based upon published reports, we anticipate that participants in the intervention arm of the study will experience a health benefit; they will be less likely to acquire TD while abroad. They also may benefit, in the event that they do acquire TD, by having a shorter duration and lesser severity of TD symptoms in comparison to those in the placebo group.

Description of the Potential Risks to Anticipated Benefit Ratio

The potential benefits of participating in this study outweigh the minimal risks.

Emergency Care

We do not anticipate participants needing emergency care as a part of this study. Participants will be provided phone and email contact information for study personnel in the event questions or concerns arise that may relate to their participation in the study.

Study Timeline

Table 1. Study Timeline, 2017–2020

Study Component	**Date of Anticipated Start**	**Date of Anticipated End**
Protocol Writing and Coordination	March 2017	October 2017
Submission of protocol/consent forms for IRB approval	November 2017	Late December 2017
Training of study personnel	January 2018	January 2018
Enrollment of participants	February 2018	July 2019
Laboratory analysis of stool specimens	February 2018	October 2019
Data entry	February 2018	October 2019
Data analysis (every 3 months)	April 2018	November 2019
Manuscript writing and clearance	November 2019	March 2020
Dissemination of findings	March 2020	

Study Population

Description and Source of the Study Population

Participants will be selected from persons seeking a pre-travel consultation at The New York Center for Travel and Tropical Medicine, New York, United States. This clinic serves travelers by providing pre-travel consultative services, in addition to post-travel screening, assessment, diagnostic, and treatment services. Centrally located in Manhattan, this clinic serves a large variety of international travelers, including tourists, immigrants or their relatives, and business travelers, among others. Travelers' destinations are equally varied, reflecting the ethnic and cultural diversity of New York City.

Definitions

Diarrhea:

≥ 3 unformed stools in 24 hours, with or without associated symptoms such as fever, cramping, or abdominal pain

Travelers' diarrhea:

Diarrhea that begins while traveling (after arrival in a country) or within 10 days after return

Study period:

Includes the 7 days prior to departure in which the pre-travel questionnaire and a biologic (stool) specimen is collected, the full duration of travel, and through 10 days maximum post-return from travel, or until both the post-travel questionnaire and submission of the post-travel biologic (stool) specimen is complete (a maximum of 17 days post-travel)

Travel companion (group):

Two or more study participants with a common relation (friends, colleagues, or relatives) with the same itinerary, who anticipate performing the same activities during travel

Trip duration:

The number of days spent in country; excludes air travel time

Severe TD:

Hospitalization to an inpatient ward either during travel or after travel with a diagnosis of diarrhea

Acquisition of AMR gene(s):

The presence in a post-travel biologic (stool) specimen of one or more antimicrobial resistance gene(s) that were not present in a pre-travel biologic (stool) specimen from the same person, and which is known to confer antimicrobial resistance to one or more antimicrobial agents ("resistome")

Medication compliance:

Full = participant takes all scheduled doses according to directions

Good = If <15% of BSS or placebo doses were missed during travel

Fair = If 15–30% of BSS or placebo doses were missed during travel

Poor = If >30% of doses were missed during travel

Questionnaire compliance:

Full = participant completes all questionnaires

Good = participant completes >80% of all during travel questionnaires, AND both the pre- and post-travel questionnaires

Fair = participant completes between 30–80% of all during travel questionnaires, including AT LEAST the pre-travel questionnaire

Poor = participant completes <40% of all during travel questionnaires, and AT LEAST the pre-travel questionnaire

Biologic (stool) specimen compliance:

Full = BOTH specimens collected and sent

Fair = ONLY one specimen collected and sent

Poor = No specimens collected and sent

Loss to follow-up:

Failure to complete the post-travel questionnaire after three attempts to contact the participant or if submission of the post-travel biologic specimen (stool) occurs more than 10 days after return from travel or is not submitted

Participant Inclusion Criteria

Willing participants must meet the inclusion criteria as outlined here:

1. Be ≥ 18 and <70 years of age at the time of enrollment
2. Sign an informed consent (see Procedures for Implementing and Documenting Informed Consent) stating willingness to participate and comply with the study protocol
3. Plan on leaving for an international trip ≥7 days after their pre-travel consultation
4. Plan on traveling in country for ≥7 days but ≤21 days (21 day limit due to BSS duration recommendations and a lack of data on longer-term BSS use) (see Definitions)
5. Traveling to either South East Asia, South Central Asia, North Africa, or Sub-Saharan Africa for at least 7 days of their itinerary (see **APPENDIX B** for list of countries classified to each region)
6. Be willing to complete an initial eligibility screening (see Recruitment and Enrollment)
7. Be willing to complete questionnaires and provide biologic specimens (stool) within 7 days of departure and within 10 days after return
8. Be willing to refrain from taking any pre-biotics, probiotics, synbiotics and/or herbal supplements throughout their study period

Participant Exclusion Criteria

Willing participants must not meet any exclusion criteria as outlined here:

1. Are <18 years of age or >69 years of age
2. Are traveling in country for <7 or >21 days (see Definitions)
3. Have known or suspected contraindications to taking BSS (including, but not limited to, travelers with kidney disease, diabetes, gout, a clotting disorder, or an allergy to any component of BSS)

4. Are pregnant (via self-report), are planning to become pregnant, or may become pregnant during travel (not actively using contraception and are sexually active), or are breastfeeding
5. Routinely take a medication known to interact with BSS (including, but not limited to, insulin, methotrexate, valproic acid, ACE inhibitors, anticoagulants, or other salicylates) (see **APPENDIX A** for complete list)
6. Have taken an antibiotic in the 30 days before departure
7. Have taken any medications that may lower one's ability to fight infection (e.g., steroids, monoclonal antibodies, etc.)
8. Have previous diagnoses of immunocompromising conditions such as HIV/AIDS, complement deficiency, immunoglobulin deficiency, or undergoing active chemotherapy or participants with chronic gastrointestinal disorders, such as chronic diarrhea, irritable bowel syndrome (IBS), inflammatory bowel disease (i.e., Crohn's disease, ulcerative colitis), celiac disease, malabsorption syndromes, pancreatic insufficiency, gallbladder disease, or current gastrointestinal cancer
9. Have had diarrhea (see Definitions) anytime in the previous 30 days, have diarrhea at the pre-travel consultation, or develop diarrhea before departure
10. Have been given doxycycline for malaria prophylaxis for the current trip (due to possible drug-drug interactions and decreased absorption of the doxycycline) (27)
11. Have an allergy to any component of the placebo tablets

Estimated Number of Participants

We anticipate enrolling approximately 500 participants (see Sample Size and Power Calculation).

Sample Size and Power Calculation

A sample size of 488 participants with full compliance to medications and questionnaires (244 participants in each arm) is needed to fulfill the objectives [anticipated incidence of the endpoint as 40% incidence of TD with a 30% minimum intervention protection (0.40-[0.4x0.30]= 0.28) with an alpha of 0.05, and 80% power].

Recruitment and Enrollment

All international travelers undergo a screening questionnaire at their pre-travel consultation to determine eligibility. This short questionnaire includes questions regarding the travelers' [see **APPENDIX A**]:

1. Age
2. Travel destination(s)
3. Trip duration
4. Acute and chronic medical conditions, including diarrhea
5. Contraindications to BSS administration
6. All current medications, including antibiotic use within the 30 days before screening
7. Willingness to participate in the trial and comply with the data collection requirements

This screening questionnaire will be administered to the traveler by study personnel at The New York Center for Travel and Tropical Medicine. The purpose will be to exclude those travelers who do not meet all inclusion criteria and those who meet any exclusion criteria (see Participant Inclusion / Exclusion Criteria). Travelers who meet the inclusion criteria and do not meet any exclusion criteria will be introduced to the project objectives and procedures, including the procedures for biologic (stool) specimen collection (see Laboratory instruments).

Once recruited and consented, eligible participants will be registered in the study at the clinic site and receive a one-page instructional handout regarding stool specimen collection and questionnaire completion [**APPENDIX K**]. All primary participant information will be kept at the clinic. Travelers will agree to inform the study personnel at their clinic site if they have a change in travel plans, begin a course of antibiotics, become pregnant before or during travel, or develop diarrhea before departure, to determine if they are still eligible to participate. In the event that a participant withdraws from the study at any time, the clinic must note this in the study database.

Description and Justification of Compensation

Gift card compensation will be provided to the participants for the time spent on this project.

The participant will receive $100 for completion of the pre-travel questionnaire and the submission of a pre-travel biologic (stool) specimen. For each daily questionnaire that the participant completes while traveling, they will receive an additional $5 (maximum additional $100). The participant will receive an additional $50 for completion of the web-based post-travel questionnaire and an additional $50 for the submission of a biologic (stool) specimen post-travel. The maximum compensation will be $300 total. The minimum participation to receive compensation is good medication compliance, good questionnaire compliance, and good biologic (stool) specimen compliance (see Definitions).

Compensation will be mailed to participants upon completion of their study period.

Statement of Extra Costs to Participants Due to their Involvement in the Study

We do not anticipate any extra costs to the participants due to their involvement.

Procedures for Implementing and Documenting Informed Consent

The New York Center for Travel and Tropical Medicine clinic must obtain and ensure scientific and ethical permission, in accordance with state and national regulations. All collaborators must adhere to strict guidelines to ensure participant confidentiality, following the Privacy Act. Biological specimens (stool) must be processed per established safety protocols.

Each participant will provide written informed consent for the project at their initial pre-travel consultation when they are enrolled by study personnel (see **APPENDIX F**). This will include (but is not limited to) information regarding the study objectives and rationale, the study procedures, how each participant's confidentiality will be protected, their rights as participants, and compensation for participation. It will also include information on how their biologic specimens will and will not be used. Study personnel will answer the travelers' questions pertaining to study logistics and BSS as necessary.

Participants may withdraw from the study at any time. Participants will self-monitor for BSS side effects and the study will be suspended in the event of safety concerns.

Plan for Monitoring the Informed Consent Process

Each informed consent will be signed in the presence of a witness to ensure all risks and benefits, in addition to an explanation of confidentiality, were described to the participant.

Variables and Interventions

Variables

Variables collected on participants are available in the **APPENDICES**. They are also described in further detail below.

Study Instruments

Questionnaires (see **APPENDICES**)

Any revisions to study procedures or instruments will be submitted to the IRB as an amendment for approval before changes are implemented.

Screening (see **APPENDIX A**)

Pre-travel (see **APPENDIX C**)

Contact information (phone and email) for each participant who consented at the pre-travel consultation/enrollment visit will be given to the study personnel who will administer the screening questionnaire. Each participant will be contacted via telephone 7 days before departure, and study personnel will review the participant's responses to each question on the screening questionnaire to ensure they are still eligible to participate. At this time, they will complete the pre-travel questionnaire with the participant on the phone and answer any questions they may have. The participant will also be reminded to submit their pre-travel biologic (stool) specimen.

Study personnel will make follow-up telephone calls on pre-departure days 7, 5, 3, and 1 as needed to ensure the questionnaire is completed.

This questionnaire will include information from the eligibility screening, in addition to the following information:

1. Demographics (e.g., age, sex, and ethnicity)
2. Medical history
3. Current medications (including use of probiotics, over-the-counter medications, or herbal supplements)
4. Previous international travel in the past 90 days
5. Travel plans including destination(s) and duration
6. Reason(s) for travel

During Travel (see **APPENDIX D**)

During travel, participants complete a daily questionnaire that will include the following information:

1. Date, time, and number of study tablets taken (to provide information on the number of missed doses)
2. Presence of gastrointestinal symptoms (e.g., abdominal cramping, fever, nausea, vomiting, tenesmus)
3. Duration of GI symptoms
4. Possible medication adverse reactions (e.g., tinnitus, black stool, etc.)
5. Other illnesses acquired (e.g., upper respiratory infection)
6. Hospital admission or contact with a healthcare facilities or providers or taking any non-routine medications for illness while abroad
7. Countries visited
8. Foods eaten (fermented foods or yogurt)

Details on the participant's trip (number 7 above) are necessary to determine risk factors for the acquisition of gut AMR genes among those participants in the placebo group vs the intervention group.

Web-based data collection will be used to facilitate data collection during travel. Each participant will receive a unique URL to input their

daily information using their unique Study ID and Group ID. A paper copy of the daily travel questionnaire will be provided as a back-up mechanism for data capture where internet access is limited or unavailable. If a participant does not think that they will have reliable internet access while abroad, they will be encouraged to use the paper copies to ensure real-time data collection. Labels will be provided to ensure that the paper copies are de-identified. Any paper forms that were completed will be returned by mail after they return from travel via a pre-paid envelope addressed to the study clinic that was given to the participant when enrolled (see Procedures).

Participants will take either BSS or placebo for the duration of travel, unless they reach a study outcome or criteria for removal (see Outcomes and Criteria for Removal from the Study).

Post-travel (see **APPENDIX E**)

Participants will be asked to submit a web-based post-travel questionnaire within 10 days after return from travel, submit a biologic (stool) specimen, and provide all daily questionnaires that were completed during travel. This will be submitted via a pre-paid envelope addressed to the study clinic that was given to the participant when enrolled.

Study personnel will make follow-up telephone calls on days 3, 5, 7, and 10 after return as needed to encourage that the post-travel questionnaire, paper questionnaires from during-travel, and biologic (stool) specimens are all submitted.

The web-based post-travel questionnaire will include questions on:

1. The development of symptoms of TD since returning from abroad, including duration of symptoms
2. New antibiotic or other medication use
3. Adverse events

4. Contact with healthcare facilities or providers
5. Changes to travel itinerary during travel

Table 2. Schedule of CLINIC events (primary and secondary objectives):

	Stages			
	Pre-travel consultation (baseline)	Pre-travel (≤7 days before departure)	During travel	Post-travel (≤10 days after return)
Screening Questionnaire	X			
Informed Consent	X			
Pre-travel Questionnaire*		X		
Follow-up call(s)		X		X†
During travel Questionnaires			X	
Post-travel Questionnaire				X
Adverse Events Reporting			X	X

*Study personnel will call the participant to complete a pre-travel questionnaire 7 days before departure and to ensure that answers to the questions on the screening questionnaire have not changed. Reminder calls will occur as per **APPENDIX G**. If the traveler is leaving exactly 7 days after the pre-travel consultation/enrollment visit, this questionnaire can be performed at the pre-travel consultation/enrollment visit.

†Study personnel will call the participant within 3 days of return from travel to remind participants to complete their web-based post-travel questionnaire, submit any paper during travel questionnaires, and collect and submit the post-travel biologic (stool) sample. Reminder calls will occur as per **APPENDIX G.**

Laboratory Instruments

All participants will collect and submit a fresh biologic (stool) sample in the 7 days before departure and within 10 days after return. Participants will be

provided all instructions and materials necessary to collect and submit these samples at enrollment. All biologic (stool) specimens will be submitted by mail, and participants will be given pre-paid shipping materials.

Participants will be provided two OMNIgene gut kits at their pre-travel consultation/enrollment visit once enrolled (see Biologic [Stool] Specimen Data Collection and Testing). These kits will come with instructions on use, handling, and shipment.

In the event that participants are able to provide a stool sample while in clinic for their pre-travel consultation/enrollment visit or during a non-study post-travel follow-up sick visit within 10 days of return, these samples will be aliquoted by study personnel at The New York Center for Travel and Tropical Medicine in the following manner:

*First, the required quantity will be placed in the participant's OMNIgene gut kit (per manufacturer directions) to be sent to CDC's Clinical and Environmental Microbiology Branch for resistome testing. The remainder will be frozen, and sent to CDC for analysis at a later date (note: OMNIgene gut kit does not allow for culture - a see Biologic [Stool] Specimen Collection and Testing). Only those participants who happen to have a biologic (stool) specimen collection completed at the clinic will have their stool analyzed by culture; this is not a requirement for participation and no additional compensation above that previously described will be offered.

Table 3. Schedule of LABORATORY events (secondary objective)

(Events from Table 2 still apply to these participants)

	Stages		
	Pre-travel	During travel	Post-travel
Biologic (stool) sample	X		X
Laboratory testing of stool	X		X

Analytic tests

Questionnaires: Data from the questionnaires will be analyzed for statistical significance comparing intervention vs placebo groups.

Biologic (stool) specimens: A highly multiplexed PCR assay detecting over 500 different AMR genes will be used to test the DNA extracted from all biologic (stool) specimens that were placed in the OMNIgene gut kit (see Biologic (Stool) Specimen Collection and Testing). Results will be analyzed to determine if the participants acquire gut AMR genes (changes to the "resistome") as a result of travel and whether or not that change varied with the bismuth subsalicylate treatment received. Additionally, stool bacteriologic culture will be performed on those specimens submitted in clinic.

Intervention

We propose the inclusion of two arms to test BSS against placebo:

1. BSS 4 tablets po bid (2.1 grams total)
2. Placebo 4 tablets po bid

Dosing will begin on the flight prior to arrival in the destination and will cease once they return home. The study medications must be taken twice daily (a morning and an evening dose); with time zone changes, the first dose should be taken to ensure that no longer than 12 hours passes before a subsequent dose.

Before departure, participants will be provided, free of charge, all needed study medication for the duration of their trip. Participants will be encouraged to take all doses of their medication and appropriately document on their daily during travel questionnaires if a dose was missed or taken off schedule.

Outcomes

The outcomes of this study will include:

	Favorable Outcome	**Non-favorable Outcome**
Hypothesis 1: Prevention of TD	Prevention of TD in the intervention group	TD in the intervention group at a rate similar to placebo; intervention drug side effects; adverse events

Hypothesis 2: Acquisition of AMR genes	Reduced or no presence of travel-associated gut AMR genes in the intervention group	No reduction in the presence of travel-associated gut AMR genes in the intervention group

The participant's study period will end in the following circumstances:

1. The traveler meets criteria for removal (see Criteria for Removal from the Study)
2. The participant voluntarily withdraws from the study (see Procedures for Implementing and Documenting Informed Consent)
3. The participant completes all study activities and submits all study questionnaires and biologic (stool) specimens
4. The participant is lost to follow-up

The participants will be instructed at enrollment to stop the study medication for any of the following reasons (although they will still be instructed to answer their questionnaires and submit biologic [stool] specimens as scheduled):

1. Antibiotics or BSS (either provided by the study personnel for TD treatment, or taken as an over-the-counter remedy) are taken for TD or for another medical condition
2. Any severe adverse events as described, such as signs and symptoms of salicylism or neurotoxicity, occur
3. Hospitalization for any reason

Barring the reasons listed above, the study medication should be taken throughout the trip duration, even if diarrhea occurs, if possible.

Criteria for Removal from the Study

Participants will be removed from the study when any of the following criteria apply:

1. Do not complete the pre-travel questionnaire (see Definitions for compliance)
2. Loss to follow-up (see Definitions)
3. Voluntary withdrawal of consent by the participant for any purpose at any time
4. Pregnancy
5. Death

6. Co-PIs decide to remove a participant because of safety or integrity of the study

In the event of removal for reason #6 above, participants will still be compensated for their participation in the study (see Description and Justification of Compensation).

The date and reason for removal will be documented in the study records.

Training for Study Personnel

All study personnel at the The New York Center for Travel and Tropical Medicine clinic will be trained in screening potential participants, questionnaire administration, and data collection. They will also be trained in appropriate informed consent practices. Laboratory study personnel at CDC's Clinical and Environmental Microbiology Branch will also be trained in the handling, preparation, storage, and testing of biologic (stool) specimens.

We do not anticipate inter-observer differences due to the structured and closed-ended format of the questionnaires and the standardization of biologic (stool) specimen collection and testing.

Data Handling and Analysis

Data Collection

Data from the screening questionnaire and pre-travel questionnaire will be collected on paper and put into REDCap by Dr. Connor's team. Data from the during travel and post-travel questionnaires will be collected electronically (web-based). Red Cap's electronic app (web-based) will be used to collect daily questionnaire data during travel, with an option to have a paper-based form if the participant prefers. Participants will be notified during consent that they should only agree to use web-based data collection during travel if they can reasonably assume that they will have reliable daily internet access; if not, they will be offered paper copies with coded labels. All data collected (either web-based or paper based) will be coded and will be entered and maintained in an electronic database and stored on a secure server by study personnel at The New York Center for Travel and Tropical Medicine clinic.

Biologic (Stool) Specimen Collection and Testing

Most biologic (stool) specimens will be self-collected by the participant using the DNA Genotek OMNIgene gut kit that will be provided to them at their pre-travel consultation/enrollment visit (each participant will receive 2 kits; one for a pre-travel biologic (stool) specimen and the other for the post-travel biologic [stool] specimen). Each kit includes detailed instructions on collection. All biologic [stool] specimens collected at home will be submitted by mail (postage pre-paid) to the travel clinic. The OMNIgene kits are stable at ambient temperatures for long periods of time and designed for home collection and postal return. Specimens will be mailed from the travel clinic to the CDC's Clinical and Environmental Microbiology Branch in batches. Biologic (stool) specimens will be stored at ambient temperature until DNA extraction.

For those participants able to provide a biologic (stool) sample during their pre-travel clinic consultation/enrollment visit or during a non-study post-travel follow-up sick visit within 10 days of return, study personnel at The New York Center for Travel and Tropical Medicine will aliquot the specimens (see Laboratory Instruments).

All biologic (stool) specimen results will be securely shared through password protected, coded data files. The Clinical and Environmental Microbiology Branch Branch will receive and share only coded information with the co-PIs (see Provision for Protecting Privacy/Confidentiality).

The following analyses will be performed on biologic (stool) specimens collected using the OMNIgene gut kit.

1. DNA extraction from single aliquots of stool
2. Highly multiplexed PCR assay detecting over 500 different antimicrobial resistance (AMR) genes will be used to test the extracted DNA
3. Amplicons produced by the panel will be sequenced on an Illumina MiSeq

Remaining biologic (stool) specimens and extracted DNA will be stored at CDC in the Clinical and Environmental Microbiology Branch indefinitely for potential further use. Stools collected at the clinic and aliquoted for freezing will be stored for potential

further use in analyses that are yet to be determined. Consent for storage of specimens for future research use will be obtained from participants.

Provision for Protecting Privacy/Confidentiality

The identity of all study participants will be protected. All questionnaires will be coded using study and group IDs and participants will each be given a unique study ID and group ID. The unique study ID will include a site letter designation of "T", year, and participant number (provided numerically from 1 to 10,000). For example, the unique identifier for the first participant would be: T2017-00001. The group ID will begin with "G" and group number (provided numerically from 1 to 10,000). Those participants who are travel companions and have identical itineraries to other participants in the study and anticipate performing the same activities, (including family and friends), are given the same group number. For example, the group ID for the first participant would be G-00001. If they have a companion traveling with them, that participant would also be G-00001.

The original completed screening questionnaires and any original completed paper daily travel forms will be housed at the clinic in a secure location and destroyed per institutional guidelines.

Biologic (stool) specimens submitted by the participant will be coded. Labels will be provided to the participants for labeling the specimens and will include the participant's unique study ID and group ID. Specimens will be sent to the CDC Clinical and Environmental Microbiology Branch by the Center for Travel and Tropical Medicine for testing and storage.

Labels will also be provided to participants for the paper copies of the during travel questionnaire to ensure confidentiality and to avoid mislabeling.

The database will be password-protected and only study personnel at New York Center for Travel and Tropical Medicine clinic will have access to the password (and thus patient identifiers). No study personnel from CDC will have access to participant identifying information. Identifying information will be stored in a secure, passcode

protected file on a password protected computer and server. This file will be destroyed upon completion of the study.

Information Management and Analysis Software

Data will be managed securely in REDCap.

SAS v9.4 will be used for analysis.

Data Entry, Editing and Management, and Data Storage

The New York Center for Travel and Tropical Medicine clinic will be responsible for managing the questionnaire data in REDCap. The data will belong to The New York Center for Travel and Tropical Medicine, will be responsible, in collaboration with partners, for the reporting of findings. Data (questionnaires) done on paper will be stored confidentially at the clinic site and electronic data will be stored confidentially on the REDCap server (see Provision for Protecting Privacy/Confidentiality). The New York Center for Travel and Tropical Medicine clinic will have full access rights and CDC will have access to data (all de-identified) only with the approval of Dr. Connor and his team.

Coded laboratory data in a password-protected database, using the participant's unique ID, will be sent from the laboratory to the New York Center for Travel and Tropical Medicine for input into REDCap.

Quality Control and Assurance

Data quality will be controlled by input of data from screening questionnaires and paper copies of the during travel questionnaires into REDCap by one or two trained personnel at The New York Center for Travel and Tropical Medicine. We will place all data, exactly as received into the database. Data from the web-based questionnaires will be input directly into the REDCap database. Data will not be altered in any way.

Laboratory quality control will be performed by the study laboratorians to ensure that all specimens are tested with appropriate controls as necessary and applicable.

Every 3 months, data entered into REDCap will be checked for completeness and accuracy by the co-PIs (CDC would only access coded data) and/or study personnel; interim data analysis will be performed every 3 months as desired.

Bias in Data Collection, Measurement, and Analysis

Double-blinded randomization will control for the introduction of selection bias or observer bias in the study; the participants and the healthcare providers will not know which arm of the study the participant is assigned to. However, our inclusion criteria necessitate the exclusion of travelers with chronic gastrointestinal diseases or travelers on certain medications, possibly creating an exclusion bias. These biases will be addressed in the limitations section of the manuscript. Participants may also introduce recall bias during the pre- and post-travel questionnaires. Reporting bias will be minimized by limiting P&G's involvement with any aspects of the study design, implementation, or analysis.

Study Limitations

As mentioned, a study limitation is the possible incorporation of exclusion bias by excluding travelers with certain chronic medical conditions and those who take certain medications. This will lead to a need to caveat the results of our study to a particular population if the intervention disproves the null hypotheses.

An additional limitation is the inability to collect biologic (stool) samples from participants while abroad in the event that they acquire TD. The collection and sending of biologic (stool) specimens over country borders is complicated and impedes us from collecting real-time potentially infectious specimens to assess the secondary objective — the direct relation between an episode of TD and the acquisition of gut AMR genes.

Participants in the intervention arm of the study may experience side effects of BSS (dark stool and dark discoloration of the mouth) due to oxidization of the bismuth component of the drug. Those in the placebo arm will likely not experience such effects, and the appearance of these effects may cause participants to suspect they were randomized into the intervention arm. Knowledge or suspicion of a participant's own study arm could influence responses to the questionnaire questions.

The results from this study may not be generalizable to all international travelers. This is due to various host factors beyond the study's control and also due to the exclusion of travelers going to regions outside of Asia and Africa. Participants are being recruited from those who are seeking specialized pre-travel medical advice. Not all travelers to the targeted regions seek this care, and travelers who seek pre-travel care may be more likely to comply with safe food/water recommendations. However, by focusing the study on a population of travelers that are visiting places with a high prevalence of TD, the study objectives are likely to be met quickly.

The laboratory assays also have limitations. The multiplex PCR assay detecting AMR genes will not provide any information regarding the organism of origin for the genes detected, including whether or not two or more genes originated from the same organism or if multiple organisms harbor the same resistance gene. This assay is only capable of detecting genes for which primers are included in the assay. However, the assay currently includes more than 500 targets and can be expanded to include additional targets of interest to study stakeholders. Also, many of the primers on this panel will readily amplify closely related gene variants even if a specific primer pair is not present, e.g. a *tetX* primer pair may amplify a *tetA* gene. Finally, the presence of a specific gene product does not guarantee the gene itself is expressed as functional antimicrobial resistance.

An additional limitation is that culture cannot be obtained from most biologic (stool) samples, making it difficult to answer future questions that involve assigning AMRs to particular organisms, unless there are further advances in sequencing/bioinformatic technology. Without a pre-screening step like the highly multiplexed PCR assay suggested, the scope of the study will have to be significantly limited to a few AMR gene groups because the real time isolation and testing of samples as they are collected is labor intensive and cannot include the breadth of targets possible with molecular testing.

Response to New or Unexpected Findings and Changes in the Study Environment

If a new or unexpected finding is identified, the co-PIs and collaborating partners will be alerted immediately. Depending on the scope and potential morbidity associated with this finding, appropriate action will be taken.

Additionally, an incident report will be filed as required by the reviewing IRB if there is an unexpected violation of study procedures or expectations or if unanticipated problems or adverse events are identified.

If deemed necessary by the IRB, we would consider using a Study Data Safety Monitoring Board to analyze the data and stop the study early in the event there are serious adverse outcomes that are significantly higher in the placebo or intervention arm. However, due to the relative safety of BSS, we do not anticipate this will be necessary.

Due to the number of participants that will need to be enrolled in this study to achieve adequate power, there is the possibility that this study may extend beyond the current study timeline. Although we do not anticipate difficulty enrolling participants, we want be prepared for any unanticipated issues with enrollment. In the event that 488 participants are not enrolled at two years, an interim analysis will be performed and additional funding will be requested to continue the project until adequate power is achieved.

Identifying, Managing, and Reporting Adverse Events

Adverse events in this study may include, but are not limited to, the following:

1. Medication side effects, such as constipation, tinnitus, or dizziness
2. Medication adverse events, such as neurologic findings from salicylate toxicity (confusion, slurred speech, drowsiness, muscle weakness, headache, mental depression) or severe stomach pain, increased sweating, or increased thirst

All participants will be asked about adverse events on each of the study questionnaires, including the daily questionnaire while traveling. If the participant experiences an

adverse event while abroad, they are to stop the study medication and contact the study personnel to report the symptoms and obtain advice.

Notifying Participants of their Individual Results

Participants will not be notified of their study results, since the results will not impact their healthcare.

Disseminating Information to the Public

The results from this study will be published in a peer-reviewed journal, however, the initial release may be in the form of an abstract for a scientific meeting.

Preliminary study findings will be shared with P&G after the data analysis phase is complete.

Budget Estimates

Table 4. CLINIC events with estimated costs (primary objective)

NOTE: Table 4 costs are for 500 participants

	Stages			
	Pre-travel consultation/ enrollment visit (baseline)	During travel	Post-travel	**TOTAL**
Recruitment materials	$200	N/A	N/A	**$200**
Printing costs (screening questionnaire, daily travel logs, labels)	$50	$150	N/A[a]	**$200**
Participant compensation	$100 per participant	up to $100 per participant ($5 per entry)	$100 per participant	**$150,000**
Advertising	$1,500	N/A	N/A	**$1,500**
IRB Costs	$3,000	N/A	N/A	**$3,000**
Prepaid envelopes for during-travel questionnaires	N/A	$1,000 ($2.00 stamp)	N/A	**$1,000**
Shipment of study medications from CDC to clinic	$10,000	N/A	N/A	**$10,000**
Administrative Personnel Costs: Physician PI (5%) plus study coordinator (40%) Recruitment/enrollment, documentation and completion of study forms and participant and data follow up	$110,000	N/A	N/A	**$110,000**
Travel (conferences)	N/A	N/A	$20,000	**$20,000**

TOTAL				**$295,900**

[a]Not applicable

Table 5. LABORATORY events with estimated costs

	Stages			
	Pre-travel consultation/enrollment visit (baseline)	During travel	Post-travel	**TOTAL**
Supplies for stool sample (OMNIgene gut kit) (n=1,000 stools or 500 participants)	$25	N/A	$25	**$25,000**
Shipping stool sample to the clinic (n=1,000 stools or 500 participants)	$10 per sample	N/A	$10 per sample	**$10,000**
Shipping stool samples to CDC lab from clinic (n=1,000 stools or 500 participants)	$15 per sample	N/A	$15 per sample	**$15,000**
Diagnostic testing of stool (n=500 stools or 250 participants)				
DNA extraction	$15	N/A	$15	**$7,500**
PCR (AMR gene assay)	$35	N/A	$35	**$17,500**
Amplicon sequencing	$25	N/A	$25	**$12,500**
Laboratorian (ORISE)	$28,000	N/A	N/A	**$28,000**
Bioinformatics (ORISE)	$66,000	N/A	N/A	**$66,000**
TOTAL				**$181,500**

Table 6. Cumulative costs of both CLINIC and LABORATORY events from Table 4 and Table 5

Cumulative totals	**Total**
Clinic Events	$295,900
Laboratory Events	$181,500
	$477,400

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Appendices

APPENDIX A: Screening Questionnaire

Screening Questionnaire

Script [read to all travelers]: Hello, my name is [*name*] and I am a [*role in the clinic*] at The New York Center for Travel and Tropical Medicine. We are conducting a study of international travelers to see if bismuth subsalicylate (or BSS) can help to prevent travelers' diarrhea. Participation in this questionnaire to see if you are eligible is voluntary, and it will only take a moment of your time. We will be collecting your email address, so in the event that you are eligible to participate, we can send you the study questionnaires and reminder emails; your email will only be used for study purposes. Are you interested in seeing if you are eligible to participate? □ YES □ NO

[If "yes", please complete the following with the traveler]:

Participant's Initials		**Date**	
Age			

☐ Participant Email (REQUIRED): _____
☐ Participant Phone #1 (OPTIONAL): _____ ☐ Participant Phone #2 (OPTIONAL):_____

1. **Are you ≥ 18 years of age and <70 years of age?**

☐ Yes*	☐ No	☐ Don't know

2. **Are you traveling to either South East Asia, South Central Asia, North Africa, or Sub-Saharan Africa?**

☐ Yes*	☐ No	☐ Don't know

3. **Are you spending at least 7 days and no more than 21 days in these regions?**

☐ Yes*	☐ No	☐ Don't know

4. **Are you leaving for your trip in at least 7 days?**

☐ Yes*	☐ No	☐ Don't know

5. **Are you now pregnant or nursing or do you plan to get pregnant before the end of your trip?**

☐ Yes	☐ No*	☐ Don't know

6. **Do you have known or suspected allergy to bismuth, salicylate, or aspirin?**

☐ Yes	☐ No*	☐ Don't know

7. **Do you have known or suspected allergy to microcrystal cellulose, calcium carbonate, artificial coloring, magnesium, mannitol, povidone, saccharin, or talc?**

☐ Yes	☐ No*	☐ Don't know

8. **Do you have kidney disease, diabetes, gout, or a clotting disorder?**

☐ Yes	☐ No*	☐ Don't know

9. **Have you taken an antibiotic in the past 30 days? For example:**
    - Doxycycline or minocycline
    - Flagyl, cotrimoxazole (e.g., Bactrim, Septra)
    - Penicillins (e.g., amoxicillin, Augmentin)
    - Cephalosporins (e.g., Keflex)
    - Fluoroquinolones (e.g., ciprofloxacin)
    - Macrolides (e.g., azithromycin [Zithromax], erythromycin)

☐ Yes	☐ No*	☐ Don't know

10. **Do you take any of the following medications or therapies?**
    - Chronic oral or intravenous IV steroids (e.g., hydrocortisone or prednisone); not inhaled steroids
    - Alkylating agents (e.g., cyclophosphamide [Cytoxan])
    - Antimetabolites (e.g., azathioprine [Imuran], 6-mercaptopurine [Purinethol])
    - Chemotherapeutic agents (e.g., including methotrexate [Trexall])
    - Tumor necrosis factor (TNF) blockers (e.g., etanercept [Enbrel], adalimumab [Humira], certolizumabpegol [Entyvio], golimumab [Simponi], and infliximab [Remicade])
    - Monoclonal antibodies (e.g., rituximab [Rituxan] or alemtuzumab [Lemtrada])
    - Insulin (e.g., Humalog, NPH, Lantus, Levemir)
    - Valproic acid (e.g., divalproex, Depakote, Depacon)
    - ACE-inhibitors (e.g., lisinopril [Zestril], enalapril [Vasotec], captopril [Capoten], ramipril [Altase], benazepril [Lotensin])
    - Anticoagulants (e.g., warfarin [Coumadin], aspirin, clopidogrel [Plavix], argatroban [Acova], bivalirudin [Angiomax], dabigatran [Pradaxa])

☐ Yes	☐ No*	☐ Don't know

11. **Do you have any of the following chronic stomach or intestinal illnesses?**
    - Irritable bowel syndrome (IBS)
    - Inflammatory bowel disease (including ulcerative colitis and Crohn's disease)
    - Celiac disease; any form of chronic diarrhea or chronic abdominal pain;
    - Pancreatic insufficiency
    - Gallbladder disease
    - Malabsorption syndromes
    - Gastrointestinal malignancy/cancer currently receiving chemo; radio, or immunotherapy
    - Chronic diarrhea from another cause

☐ Yes	☐ No*	☐ Don't know

12. Do you have any illnesses that you have been told lowers your body's ability to fight infection?

- Missing or non-functioning spleen
- Insulin-dependent diabetes mellitus
- Diabetes mellitus receiving oral hypoglycemic medications (e.g. metformin, glyburide)
- HIV/AIDS
- Other immunodeficiency syndrome (e.g. complement deficiency, antibody or immunoglobulin deficiency)
- Malignancy or cancer under active chemo, radio, or immunotherapy; or any other immunocompromising condition?

☐ Yes	☐ No*	☐ Don't know

13. Have you ever received a solid organ transplant?

☐ Yes	☐ No*	☐ Don't know

14. Have you had diarrhea (3 or more unformed stools in 24 hours) in the 30 days before enrollment or do you currently have diarrhea?

☐ Yes	☐ No*	☐ Don't know

**Must answer "Yes" to questions 1, 2, 3, AND 4 and "No" to all subsequent to be eligible for enrollment.*

☐ Eligible per Appx A	☐ Not Eligible per Appx A	Comments:

By signing below, I confirm that I conducted the verbal screening and have reviewed the answers before proceeding with additional study activities:

Name of Interviewer		**Signature**	
Date			

If eligible, collect the following:

Participant First Name: _____ Participant Last Name: _____
State of Residence: _____ County of Residence: _____
Residence Zip Code: _____

Generation of Study ID and Group ID:

UNIQUE STUDY ID:

The unique study ID will include a site letter designation of "T", year, and participant number (provided numerically from 1 to 10,000).

*For example, the unique identifier for the first participant would be: T2017-00001.

GROUP ID:

The group ID will begin with "G" and a group number (provided numerically from 1 to 10,000). Those participants who are travel companions and have identical itineraries to other participants in the study

and anticipate performing the same activities, (including family and friends), are given the same number.

*For example, the group ID for the first participant would be G-00001. Their mother, traveling with them, would also be G-00001.

Study ID Number	

APPENDIX B: Country Listing for Inclusion

South East Asia

Cambodia
Laos
West Malaysia (excluding Malaysian Borneo)
Myanmar (Burma)
Thailand
Vietnam

South Central Asia

Afghanistan
Bangladesh
Bhutan
India
Maldives
Nepal
Pakistan
Sri Lanka

North Africa

Algeria
Egypt
Libya
Morocco
Sudan
Tunisia

Sub-Saharan Africa (*Does NOT include the country of SOUTH AFRICA*)

Angola	Congo	Guinea-Bissou	Mozambique	Sierra Leone
Benin	Democratic Republic of the Congo	Kenya	Namibia	Somalia
Botswana	Côte d' Ivoire	Lesotho	Niger	South Sudan
Burkina Faso	Djibouti	Liberia	Nigeria	Swaziland
Burundi	Equatorial Guinea	Madagascar	Reunion	United Republic of Tanzania
Cameroon	Eritrea	Malawi	Rwanda	The Gambia
Cabo (Cape) Verde	Ethiopia	Mali	Saint Helena	Togo
Central African Republic	Gabon	Mauritania	São Tomé and Príncipe	Uganda
Chad	Ghana	Mauritius	Senegal	Zambia
Comoros	Guinea	Mayotte	Seychelles	Zimbabwe

APPENDIX C: Pre-travel Questionnaire

Pre-travel Questionnaire

PARTICIPANT UNIQUE ID:_____
GROUP ID: _____

[Please review the screening questionnaire with the participant.]
Have there been any changes to the screening questionnaire making the participant ineligible to participate?

a. Yes
b. No

If yes, please describe:

_____

Demographics

Age (in years): _____
Sex (M/F): _____
Race:

a. White or Caucasian
b. Black or African American
c. American Indian or Alaskan native
d. Asian or Pacific Islander
e. Other (please specify) _____
f. Don't know
g. I would rather not answer

Ethnicity:

a. Hispanic
b. Non-Hispanic

Medical History

2. Do you have any medical conditions or illnesses?

a. Yes
b. No
c. Don't know
d. I would rather not answer

If yes to question 1, please list them all.

_____

3. **Do you have recurrent or persistent tinnitus (ringing in the ears)?**
    a. Yes
    b. No
    c. Don't know
    d. I would rather not answer

4. **Do you take any medications on a regular basis?**
    a. Yes
    b. No
    c. Don't know
    d. I would rather not answer

If Yes to question 3, please list them all:

1.	4.
2.	5.
3.	6.

5. **Have you taken any oral or intravenous (IV) antibiotics in the past 90 days for any reason** (e.g., azithromycin [Zithro-max], ciprofloxacin [Cipro], rifaximin [Xifaxan], doxycycline or minocycline, metronidazole [Flagyl], cotrimoxazole [Bactrim or Septra], penicillins [e.g., amoxicillin or Augmentin], or cephalosporins [e.g., Keflex])?
    a. Yes
    b. No
    c. Don't know
    d. I would rather not answer

If yes to question 4, please give the antibiotic name, start date, duration, and illness taken for:

No.	Antibiotic Name	Start Date (MM/DD/YYYY)	Duration (Days)	Illness taken for
1				
2				
3				
4				
5				
6				

6. **In the past 30 days, have you taken a probiotic?** -
Probiotics are live microorganisms (such as certain types of bacteria) that may benefit the health of the person consuming them. Yogurts and other fermented dairy products are probiotics. Probiotics can also take the form of capsules, pills, or powders. Probiotics also include any foods or drinks labeled as containing "live and active cultures" or "probiotics."
    a. Yes

b. No
c. Don't know
d. I would rather not answer

Travel History

7. Have you traveled internationally in the past 90 days?

a. Yes
b. No
c. Don't know
d. I would rather not answer

If yes to question 6, which countries did you travel to and what were your dates of travel? If the exact dates of travel are unknown, please list the month and year only.

Country name	Dates of travel (MM/DD/YY to MM/DD/YY)
1.	__/__/__ to __/__/__ ☐ Unknown
2.	__/__/__ to __/__/__ ☐ Unknown
3.	__/__/__ to __/__/__ ☐ Unknown
4.	__/__/__ to __/__/__ ☐ Unknown
5.	__/__/__ to __/__/__ ☐ Unknown
6.	__/__/__ to __/__/__ ☐ Unknown

8. Where are you planning to travel? Please specify each country name, the specific locations you plan to visit, and the number of days you plan to spend in each country.

Country name	Specific location(s) to be visited (e.g., city/town, attraction)	Number of days in this country
1.		
2.		
3.		

4.		
5.		
6.		

9. What is the total duration of your trip (in days)?

_____

10. What is your <u>main</u> reason for travel (circle only one)?

a. Education or research (includes medical or veterinary-type education)
b. Adoption
c. Business
d. Leisure/tourism
e. Immigrant traveling back to visit friends or relatives
f. Providing medical care as a health professional (including paid or unpaid/volunteer)
g. Receiving medical care (as a patient)
h. Non-medical service or volunteer work
i. Missionary work
j. Military service
k. Attending large gathering or event (e.g., large sporting event, large conference, Hajj)
l. Adventuring (extreme mountaineering, water sports, outdoor activities, etc.)
m. Other:

_____

n. I would rather not answer

11. What are the <u>secondary</u> reasons for travel (circle all that apply)?

a. Education or research (includes medical or veterinary-type education)
b. Adoption
c. Business
d. Leisure/tourism
e. Returning to region of origin of self or family to visit friends and relatives
f. Providing medical care as a health professional (including paid or unpaid/volunteer)
g. Receiving medical care (as a patient)
h. Non-medical service or volunteer work
i. Missionary work
j. Military service
k. Attending large gathering or event (e.g., large sporting event, large conference, Hajj)
l. Adventuring (extreme mountaineering, water sports, outdoor activities, etc.)
m. Other:

_____

n. I would rather not answer

This survey is now completed.

Thank you very much for your time.

APPENDIX D: During-travel Questionnaire

During Travel Questionnaire

PARTICIPANT UNIQUE ID:_____

GROUP ID: _____

	Day 1	Day 2	Day 3	Day 4	Day 5
Please write the calendar date that corresponds to each day of your trip. Day 1 is your first day of arrival in the country.					
Number of tablets taken in the morning (maximum 4)					
Number of tablets taken in the afternoon/evening (maximum 4)					
Symptoms in the prior 24 hours (check off all that apply)	. . .	. . .	. . .	. . .	. . .
Fever	☐	☐	☐	☐	☐
Nausea	☐	☐	☐	☐	☐
Vomiting	☐	☐	☐	☐	☐
Loose stools (less than three in a 24 hour period)	☐	☐	☐	☐	☐
Diarrhea (three or more loose stools in 24 hours)	☐	☐	☐	☐	☐
Bloody diarrhea	☐	☐	☐	☐	☐
Constipation	☐	☐	☐	☐	☐
Dark or black stool	☐	☐	☐	☐	☐
Dark or black tongue	☐	☐	☐	☐	☐
Abdominal pain	☐	☐	☐	☐	☐
Abdominal cramps	☐	☐	☐	☐	☐
Urgency to relieve oneself (in reference to stool)	☐	☐	☐	☐	☐
Bloating	☐	☐	☐	☐	☐
Flatulence/gas	☐	☐	☐	☐	☐
Congestion/runny nose	☐	☐	☐	☐	☐
Sore throat	☐	☐	☐	☐	☐
Cough	☐	☐	☐	☐	☐
Rash	☐	☐	☐	☐	☐
Headache	☐	☐	☐	☐	☐
Dizziness	☐	☐	☐	☐	☐
Ringing in your ears	☐	☐	☐	☐	☐
Muscle pain/soreness	☐	☐	☐	☐	☐
Joint pain/swelling/redness	☐	☐	☐	☐	☐

Drowsiness (severe)	☐	☐	☐	☐	☐
Confusion	☐	☐	☐	☐	☐
Depression	☐	☐	☐	☐	☐
Other (list/describe):	☐	☐	☐	☐	☐
Medical care	. . .	. . .	. . .	. . .	. . .
Did you seek medical care for one or more of these symptoms? (**Y**es/**N**o/**D**on't Know)	Y N D	Y N D	Y N D	Y N D	Y N D
➔ If yes, where? (check the corresponding box(es))	. . .	. . .	. . .	. . .	. . .
A doctor's office					
Urgent care clinic (outpatient)					
Emergency room					
Hospital outpatient clinic setting					
Hospital inpatient ward – hospitalized					
Other (specify)					
➔ Was your diagnosis travelers' diarrhea? (**Y**es/**N**o/**D**on't Know)	Y N D	Y N D	Y N D	Y N D	Y N D
Have you started any medications in the past 24 hours? (**Y**es/**N**o/**D**on't Know)	Y N D	Y N D	Y N D	Y N D	Y N D
➔ If yes, what medication(s)?					
➔ Please circle those that were provided for you at your pre-travel consultation with the study clinic.	. . .	. . .	. . .	. . .	. . .
What city(ies) and country(ies) have you been in during the past 24 hours? Please write answers to all questions in the corresponding box.					
Have you eaten any of the following in the past 24 hours? (**Y**es/**N**o/**D**on't Know)	. . .	. . .	. . .	. . .	. . .
➔ Fermented or pickled foods (*e.g.*, bean paste, fish kimchi, etc.)	Y N D	Y N D	Y N D	Y N D	Y N D
➔ Yogurt	Y N D	Y N D	Y N D	Y N D	Y N D
Did you have any diarrhea between completing the pre-travel questionnaire and arriving in the country? (**Y**es/**N**o/**D**on't Know)	Y N D	. . .	. . .	. . .	. . .
Comments:					

PARTICIPANT UNIQUE ID:_____

GROUP ID: _____

	Day 6	Day 7	Day 8	Day 9	Day 10
Please write the calendar date that corresponds to each day of your trip. Day 1 is your first day of arrival in the country.					
Number of tablets taken in the morning (maximum 4)					
Number of tablets taken in the afternoon/evening (maximum 4)					
Symptoms in the prior 24 hours (check off all that apply)	. . .	. . .	. . .	. . .	. . .
Fever	☐	☐	☐	☐	☐
Nausea	☐	☐	☐	☐	☐
Vomiting	☐	☐	☐	☐	☐
Loose stools (less than three in a 24 hour period)	☐	☐	☐	☐	☐
Diarrhea (three or more loose stools in 24 hours)	☐	☐	☐	☐	☐
Bloody diarrhea	☐	☐	☐	☐	☐
Constipation	☐	☐	☐	☐	☐
Dark or black stool	☐	☐	☐	☐	☐
Dark or black tongue	☐	☐	☐	☐	☐
Abdominal pain	☐	☐	☐	☐	☐
Abdominal cramps	☐	☐	☐	☐	☐
Urgency to relieve oneself (in reference to stool)	☐	☐	☐	☐	☐
Bloating	☐	☐	☐	☐	☐
Flatulence/gas	☐	☐	☐	☐	☐
Congestion/runny nose	☐	☐	☐	☐	☐
Sore throat	☐	☐	☐	☐	☐
Cough	☐	☐	☐	☐	☐
Rash	☐	☐	☐	☐	☐
Headache	☐	☐	☐	☐	☐
Dizziness	☐	☐	☐	☐	☐
Ringing in your ears	☐	☐	☐	☐	☐
Muscle pain/soreness	☐	☐	☐	☐	☐
Joint pain/swelling/redness	☐	☐	☐	☐	☐
Drowsiness (severe)	☐	☐	☐	☐	☐
Confusion	☐	☐	☐	☐	☐
Depression	☐	☐	☐	☐	☐

Other (list/describe):	☐	☐	☐	☐	☐
Medical care	. . .	. . .	. . .	. . .	. . .
Did you seek medical care for one or more of these symptoms? (**Y**es/**N**o/**D**on't Know)	Y N D	Y N D	Y N D	Y N D	Y N D
➔ If yes, where? (check the corresponding box(es))	. . .	. . .	. . .	. . .	. . .
A doctor's office					
Urgent care clinic (outpatient)					
Emergency room					
Hospital outpatient clinic setting					
Hospital inpatient ward – hospitalized					
Other (specify)					
➔ Was your diagnosis travelers' diarrhea? (**Y**es/**N**o/**D**on't Know)	Y N D	Y N D	Y N D	Y N D	Y N D
Have you started any medications in the past 24 hours? (**Y**es/**N**o/**D**on't Know)	Y N D	Y N D	Y N D	Y N D	Y N D
➔ If yes, what medication(s)?					
➔ Please circle those that were provided for you at your pre-travel consultation with the study clinic.	. . .	. . .	. . .	. . .	. . .
What city(ies) and country(ies) have you been in during the past 24 hours? Please write answers to all questions in the corresponding box.					
Have you eaten any of the following in the past 24 hours? (**Y**es/**N**o/**D**on't Know)	. . .	. . .	. . .	. . .	. . .
➔ Fermented or pickled foods (*e.g.*, bean paste, fish kimchi, etc.)	Y N D	Y N D	Y N D	Y N D	Y N D
➔ Yogurt	Y N D	Y N D	Y N D	Y N D	Y N D
Comments:					

PARTICIPANT UNIQUE ID:_____

GROUP ID: _____

	Day 11	Day 12	Day 13	Day 14	Day 15
Please write the calendar date that corresponds to each day of your trip. Day 1 is your first day of arrival in the country.					
Number of tablets taken in the morning (maximum 4)					
Number of tablets taken in the afternoon/evening (maximum 4)					
Symptoms in the prior 24 hours (check off all that apply)	. . .	. . .	. . .	. . .	. . .
Fever	☐	☐	☐	☐	☐
Nausea	☐	☐	☐	☐	☐
Vomiting	☐	☐	☐	☐	☐
Loose stools (less than three in a 24 hour period)	☐	☐	☐	☐	☐
Diarrhea (three or more loose stools in 24 hours)	☐	☐	☐	☐	☐
Bloody diarrhea	☐	☐	☐	☐	☐
Constipation	☐	☐	☐	☐	☐
Dark or black stool	☐	☐	☐	☐	☐
Dark or black tongue	☐	☐	☐	☐	☐
Abdominal pain	☐	☐	☐	☐	☐
Abdominal cramps	☐	☐	☐	☐	☐
Urgency to relieve oneself (in reference to stool)	☐	☐	☐	☐	☐
Bloating	☐	☐	☐	☐	☐
Flatulence/gas	☐	☐	☐	☐	☐
Congestion/runny nose	☐	☐	☐	☐	☐
Sore throat	☐	☐	☐	☐	☐
Cough	☐	☐	☐	☐	☐
Rash	☐	☐	☐	☐	☐
Headache	☐	☐	☐	☐	☐
Dizziness	☐	☐	☐	☐	☐
Ringing in your ears	☐	☐	☐	☐	☐
Muscle pain/soreness	☐	☐	☐	☐	☐
Joint pain/swelling/redness	☐	☐	☐	☐	☐
Drowsiness (severe)	☐	☐	☐	☐	☐
Confusion	☐	☐	☐	☐	☐
Depression	☐	☐	☐	☐	☐

Other (list/describe):	☐	☐	☐	☐	☐
Medical care	. . .	. . .	. . .	. . .	. . .
Did you seek medical care for one or more of these symptoms? (**Y**es/**N**o/**D**on't Know)	Y N D	Y N D	Y N D	Y N D	Y N D
➔ If yes, where? (check the corresponding box(es))	. . .	. . .	. . .	. . .	. . .
A doctor's office					
Urgent care clinic (outpatient)					
Emergency room					
Hospital outpatient clinic setting					
Hospital inpatient ward – hospitalized					
Other (specify)					
➔ Was your diagnosis travelers' diarrhea? (**Y**es/**N**o/**D**on't Know)	Y N D	Y N D	Y N D	Y N D	Y N D
Have you started any medications in the past 24 hours? (**Y**es/**N**o/**D**on't Know)	Y N D	Y N D	Y N D	Y N D	Y N D
➔ If yes, what medication(s)?					
➔ Please circle those that were provided for you at your pre-travel consultation with the study clinic.	. . .	. . .	. . .	. . .	. . .
What city(ies) and country(ies) have you been in during the past 24 hours? Please write answers to all questions in the corresponding box.					
Have you eaten any of the following in the past 24 hours? (**Y**es/**N**o/**D**on't Know)	. . .	. . .	. . .	. . .	. . .
➔ Fermented or pickled foods (*e.g.*, bean paste, fish kimchi, etc.)	Y N D	Y N D	Y N D	Y N D	Y N D
➔ Yogurt	Y N D	Y N D	Y N D	Y N D	Y N D
Comments:					

PARTICIPANT UNIQUE ID:_____

GROUP ID: _____

	Day 16	Day 17	Day 18	Day 19	Day 20
Please write the calendar date that corresponds to each day of your trip. Day 1 is your first day of arrival in the country.					
Number of tablets taken in the morning (maximum 4)					
Number of tablets taken in the afternoon/evening (maximum 4)					
Symptoms in the prior 24 hours (check off all that apply)	. . .	. . .	. . .	. . .	. . .
Fever	☐	☐	☐	☐	☐
Nausea	☐	☐	☐	☐	☐
Vomiting	☐	☐	☐	☐	☐
Loose stools (less than three in a 24 hour period)	☐	☐	☐	☐	☐
Diarrhea (three or more loose stools in 24 hours)	☐	☐	☐	☐	☐
Bloody diarrhea	☐	☐	☐	☐	☐
Constipation	☐	☐	☐	☐	☐
Dark or black stool	☐	☐	☐	☐	☐
Dark or black tongue	☐	☐	☐	☐	☐
Abdominal pain	☐	☐	☐	☐	☐
Abdominal cramps	☐	☐	☐	☐	☐
Urgency to relieve oneself (in reference to stool)	☐	☐	☐	☐	☐
Bloating	☐	☐	☐	☐	☐
Flatulence/gas	☐	☐	☐	☐	☐
Congestion/runny nose	☐	☐	☐	☐	☐
Sore throat	☐	☐	☐	☐	☐
Cough	☐	☐	☐	☐	☐
Rash	☐	☐	☐	☐	☐
Headache	☐	☐	☐	☐	☐
Dizziness	☐	☐	☐	☐	☐
Ringing in your ears	☐	☐	☐	☐	☐
Muscle pain/soreness	☐	☐	☐	☐	☐
Joint pain/swelling/redness	☐	☐	☐	☐	☐
Drowsiness (severe)	☐	☐	☐	☐	☐
Confusion	☐	☐	☐	☐	☐
Depression	☐	☐	☐	☐	☐

Other (list/describe):	☐	☐	☐	☐	☐
Medical care	. . .	. . .	. . .	. . .	. . .
Did you seek medical care for one or more of these symptoms? (**Y**es/**N**o/**D**on't Know)	Y N D	Y N D	Y N D	Y N D	Y N D
➔ If yes, where? (check the corresponding box(es))	. . .	. . .	. . .	. . .	. . .
A doctor's office					
Urgent care clinic (outpatient)					
Emergency room					
Hospital outpatient clinic setting					
Hospital inpatient ward – hospitalized					
Other (specify)					
➔ Was your diagnosis travelers' diarrhea? (**Y**es/**N**o/**D**on't Know)	Y N D	Y N D	Y N D	Y N D	Y N D
Have you started any medications in the past 24 hours? (**Y**es/**N**o/**D**on't Know)	Y N D	Y N D	Y N D	Y N D	Y N D
➔ If yes, what medication(s)?					
➔ Please circle those that were provided for you at your pre-travel consultation with the study clinic.	. . .	. . .	. . .	. . .	. . .
What city(ies) and country(ies) have you been in during the past 24 hours? Please write answers to all questions in the corresponding box.					
Have you eaten any of the following in the past 24 hours? (**Y**es/**N**o/**D**on't Know)	. . .	. . .	. . .	. . .	. . .
➔ Fermented or pickled foods (*e.g.*, bean paste, fish kimchi, etc.)	Y N D	Y N D	Y N D	Y N D	Y N D
➔ Yogurt	Y N D	Y N D	Y N D	Y N D	Y N D
Comments:					

PARTICIPANT UNIQUE ID:_____

GROUP ID: _____

	Day 21
Please write the calendar date that corresponds to each day of your trip. Day 1 is your first day of arrival in the country.	
Number of tablets taken in the morning (maximum 4)	
Number of tablets taken in the afternoon/evening (maximum 4)	
Symptoms in the prior 24 hours (check off all that apply)	. . .
Fever	☐
Nausea	☐
Vomiting	☐
Loose stools (less than three in a 24 hour period)	☐
Diarrhea (three or more loose stools in 24 hours)	☐
Bloody diarrhea	☐
Constipation	☐
Dark or black stool	☐
Dark or black tongue	☐
Abdominal pain	☐
Abdominal cramps	☐
Urgency to relieve oneself (in reference to stool)	☐
Bloating	☐
Flatulence/gas	☐
Congestion/runny nose	☐
Sore throat	☐
Cough	☐
Rash	☐
Headache	☐
Dizziness	☐
Ringing in your ears	☐
Muscle pain/soreness	☐
Joint pain/swelling/redness	☐

Drowsiness (severe)	☐
Confusion	☐
Depression	☐
Other (list/describe):	☐
Medical care	. . .
Did you seek medical care for one or more of these symptoms? (**Y**es/**N**o/**D**on't Know)	Y N D
➔ If yes, where? (check the corresponding box(es))	. . .
A doctor's office	
Urgent care clinic (outpatient)	
Emergency room	
Hospital outpatient clinic setting	
Hospital inpatient ward – hospitalized	
Other (specify)	
➔ Was your diagnosis travelers' diarrhea? (**Y**es/**N**o/**D**on't Know)	Y N D
Have you started any medications in the past 24 hours? (**Y**es/**N**o/**D**on't Know)	Y N D
➔ If yes, what medication(s)?	
➔ Please circle those that were provided for you at your pre-travel consultation with the study clinic.	. . .
What city(ies) and country(ies) have you been in during the past 24 hours? Please write answers to all questions in the corresponding box.	
Have you eaten any of the following in the past 24 hours? (**Y**es/**N**o/**D**on't Know)	. . .
➔ Fermented or pickled foods (*e.g.*, bean paste, fish kimchi, etc.)	Y N D
➔ Yogurt	Y N D
Comments:	

APPENDIX E: Post-travel Questionnaire

Post-travel Questionnaire

PARTICIPANT UNIQUE ID:_____
GROUP ID: _____

1. **Have you had any of the following symptoms since returning home from your trip (circle all that apply)?**

Symptom	Duration (in days)
Fever	
Nausea	
Vomiting	
Loose stools (less than three in a 24 hour period)	
Diarrhea (three or more loose stools in 24 hours)	
Bloody diarrhea	
Constipation	
Dark or black stool	
Dark or black tongue	
Abdominal pain	
Abdominal cramps	
Fecal urgency	
Bloating	
Flatulence/gas	
Congestion/runny nose	
Sore throat	
Cough	
Rash	
Headache	
Dizziness	
Ringing in your ears	
Muscle pain/soreness	
Joint pain/swelling/redness	
Drowsiness (severe)	
Confusion	
Depression	
Other (list/describe):	
Don't know	. . .
I would rather not answer	. . .

2. **Did you start taking any of the following medications since you arrived home (circle all that apply)?**

   a. **Oral or intravenous (IV) antibiotics** (e.g., azithromycin [Zithromax], ciprofloxacin [Cipro], rifaximin [Xifaxan], doxycycline or minocycline, metronidazole [Flagyl], cotrimoxazole [Bactrim or Septra], penicillins [e.g., amoxicillin, Augmentin], or cephalosporins [e.g., Keflex])
   b. **Antidiarrheals** (e.g., bismuth subsalicylate [Pepto Bismol/Kaopectate])
   c. **Antimotility agents** (e.g., loperamide [Imodium])
   d. **H2 blocker** (e.g., ranitidine [Zantac], cimetidine [Tagamet], famotidine [Pepcid])
   e. **Proton pump inhibitor** (e.g., omeprazole [Prilosec], pantoprazole [Protonix], lansoprazole [Prevacid], esomeprazole [Nexium])
   f. **Other antacids** (e.g., calcium carbonate [Tums/Rolaids], Maalox/Mylanta)
   g. **Probiotics** (*Yogurts and other fermented dairy products are probiotics. Probiotics can also take the form of capsules, pills, or powders. Probiotics also include any foods or drinks labeled as containing "live and active cultures" or "probiotics"*)
   h. **Oral or intravenous steroids** (e.g., prednisone, hydrocortisone)
   i. **Local/natural or herbal remedies** (herbs, etc.)
   j. **Other:**

      _____

3. **Did you experience any of the following at any point during your travels?**

Symptom	(**Y**es/**N**o/**D**on't Know)	**If yes, what was the duration (in days)?**
Ringing in your ears	Y N D	
Constipation	Y N D	
Dark or black stool	Y N D	
Dark or black tongue	Y N D	
Drowsiness	Y N D	
Nausea	Y N D	
Vomiting	Y N D	
Rash	Y N D	
Other (list/describe):		

4. **Did you seek medical care for an illness since returning to the United States after your trip?**
   a. Yes
   b. No
   c. Don't know
   d. I would rather not answer

   If yes to question 4, where did you seek and receive care?
   a. A doctor's office
   b. Urgent care clinic (outpatient)
   c. Emergency room
   d. Hospital outpatient clinical setting
   e. Hospital inpatient ward – hospitalized
   f. Other (please specify)

      _____

   If yes to question 4, what was/were your diagnosis/es?

_____
_____

If yes to question 4, were you told that your illness was related to travel?

a. Yes
b. No
c. Don't know
d. I would rather not answer

If yes to question 4, were you told that you had travelers' diarrhea?

a. Yes
b. No
c. Don't know
d. I would rather not answer

If yes to question 4, were you told that you had a bacteria that was resistant to antibiotics?

a. Yes
b. No
c. Don't know
d. I would rather not answer

5. Did you travel to any countries not discussed during your pre-travel visit?

a. Yes
b. No
c. Don't know
d. I would rather not answer

If yes to question 5, please list the new countries you visited, the location(s) visited, and the number of days in each country:

Country name	Specific location(s) to be visited (e.g., city/town, attraction)	Number of days in this country
1.		
2.		
3.		
4.		
5.		

6. Did you not travel to a country you discussed at your pre-travel visit?

a. Yes

b. No
c. Don't know
d. I would rather not answer

If yes to question 6, please list the countries you did not visit.

Country name
1.
2.
3.
4.
5.

7. Did the time you spent in each country change from what you reported at your pre-travel visit?

a. Yes
b. No
c. Don't know
d. I would rather not answer

If yes to question 7, please list the countries in which the amount of time spent changed, including the number of days actually spent in each country:

Country name	Number of days in this country
1.	
2.	
3.	
4.	
5.	

This survey is now completed.

Thank you very much for your time.

APPENDIX F: Informed Consent Form

[PLEASE SEE SEPARATE DOCUMENT]

APPENDIX G: Follow-up Phone Call Schedule

	7 days before travel	5 days before travel	3 days before travel	1 day before travel
Review all screening questionnaire answers with the participant, perform the pre-travel questionnaire, and provide a reminder for stool sample submission	X	X	X	X
	3 days after travel	5 days after travel	7 days after travel	10 days after travel
Reminder for post-travel questionnaire and stool sample submission	X	X	X	X

APPENDIX H: Promotion Materials

Facebook:

Traveling overseas? Our clinic is offering an opportunity to enroll in a study regarding travelers diarrhea. You'll be compensated for your participation. Check out our website at www.travelhealth.net for more information!

Traveling abroad? Consider enrolling in our study regarding travelers' diarrhea. Visit our website at www.travelhealth.net or schedule an appointment by calling 646-374-4132 to learn more!

Participate in our study regarding travelers' diarrhea! If you're interested and have international travel plans, check out our website at www.travelhealth.net for more information.

Twitter (@nyctravelmed):

Traveling abroad? Enroll in our study regarding travelers' diarrhea. More info at www.travelhealth.net! [102 characters]

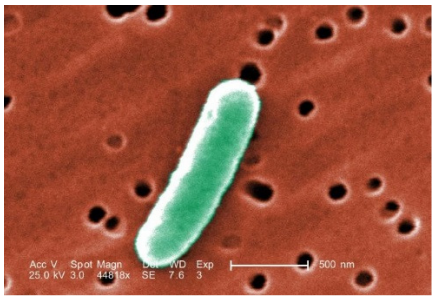
Make $ during travel & help us learn more - enroll in a study regarding travelers' diarrhea. Visit www.travelhealth.net! [121 characters]

Our clinic is now enrolling for a study regarding travelers' diarrhea. Make $ and learn more at www.travelhealth.net! [117 characters]

Instagram:

[stock image from public health image library]

Participate in a study regarding travelers' diarrhea! Visit our website at www.travelhealth.net for more info on how to participate!



[stock image from public health image library]

Traveling internationally? Participate in a study regarding travelers' diarrhea and receive compensation for your time! Visit our website at www.travelhealth.net for more info!

Clinic website:

Participate in a Study to Prevent Travelers' Diarrhea

The New York Travel and Tropical Medicine Clinic is now enrolling international travelers in a study of a medication to prevent travelers' diarrhea. Participants will be compensated for their time. If you or someone you know is 18 or older, is traveling internationally in the upcoming months, and is interested in learning more, give us a call or schedule an appointment with a provider.

Newspaper (Manhattan and/or Brooklyn):

Traveling internationally soon?

The New York Travel and Tropical Medicine Clinic is now enrolling international travelers in a study of a medication to prevent travelers' diarrhea. Participants will be compensated for their time! If you or someone you know is 18 or older, is traveling internationally in the upcoming months, and is interested in learning more, give us a call at 646-374-4132 to schedule an appointment. Visit us at www.travelhealth.net.

Social media management plan:

1. User comments will be monitored for accuracy weekly
2. For any technical issues regarding posts on Facebook, Twitter, and Instagram, those platforms will be notified of issues. A web management organization assists with the clinic website.
3. Inappropriate or misleading comments will be addressed immediately. Depending on the nature of the comment, it will be addressed in public forum or removed and addressed specifically with the individual who posted the comment.

APPENDIX I: Data Analysis Table Shells

Table 1. Baseline Characteristics of Participants

	BSS	Placebo	P-value
Demographics			
Age			
Sex			
Race			
Ethnicity			
Medical History			
Chronic medical conditions			
Tinnitis			
Antibiotics between 30 to 90 days prior to travel			
Probiotics within 30 days			
Travel			
Duration of travel			
Primary reason for travel			
Secondary reasons for travel			
Exposures during travel			
Fermented or pickled foods			
Yogurt			
Attended provider (by type)			

Table 2. Odds ratios (OR) of TD associated with BSS among international travelers, adjusting for select factors

	Total, N	TD, %	OR (95% CI)
Tablets			
BSS			
Placebo			Ref
Age groups, years			
≥18			
Sex			
Male			
Female			Ref
Race/ethnicity			
.			
.			
Travel region			
Travel duration			

Table 3. The proportion of participants having resistance genes before and after travel; acquiring, losing, or maintaining genes after travel

	All participants	BSS	Placebo	P-value
Pre-travel				
Gene 1				
Gene 2				
Gene 3				
Gene 4				
…				
Post-travel				
Gene 1				
Gene 2				
Gene 3				
Gene 4				
…				
Acquisition of gene				
Gene 1				
Gene 2				
Gene 3				
Gene 4				
…				
Loss of gene				
Gene 1				
Gene 2				
Gene 3				
Gene 4				
…				
Maintenance of gene				
Gene 1				
Gene 2				
Gene 3				
Gene 4				
…				

APPENDIX J: Auto-Generate Email for Questionnaires

Greetings,

It's time to complete your "Day X" survey!

If your travel plans change or you need to contact us regarding the study, please contact us at: NYTravelStudy@gmail.com.

Thank you,
The New York Center for Travel and Tropical Medicine

You may open the survey in your web browser by clicking the link below:
Bismuth subsalicylate's Role in the Prevention of Travelers' Diarrhea: Daily Questionnaire

If the link above does not work, try copying the link below into your web browser:
https://rdcp.cdc.gov/surveys/?s=QbH5omcTYB

This link is unique to you and should not be forwarded to others.

APPENDIX K: Handout: Participant Instructions

Thank you for your participation in this study to see if bismuth subsalicylate (BSS) prevents travelers' diarrhea or prevents you from being colonized with drug-resistant bacteria in your gut.

Summary of Procedures for Participants:

- Approximately **7 days prior to your departure**, you will receive a call from clinic personnel. They will verify that the responses you provided to the brief questionnaire at your enrollment visit are still accurate. They will also administer a **Pre-Travel Questionnaire** over the phone and remind you to **submit your first stool sample**.
    - After this call, you will receive an email containing your Study ID, Group ID, and a link for you to fill out some addition information about your upcoming trip.
    - You **MUST** complete this questionnaire prior to departing for your trip. ***If you do not complete it, you will be excluded from the study.***
    - ***If you do not receive an email within 24 hours of completing the enrollment form, please contact the travel clinic.***
    - Stool sample mailing materials will be provided to you at your pre-travel visit. ***If they were not received or if they were misplaced, please contact the travel clinic.***
- You will receive your first **Daily Travel Log** via email on your first day of travel in Africa (excluding South Africa) and/or South East or South Central Asia. You will continue to receive these emails daily for the duration of your travels in these regions.
    - Complete the Daily Travel Log after taking your second dose of tablets (*e.g.*, evening).
    - You will have 48 hours to complete each Daily Travel Log entry. You will receive an email reminder every 12 hours until each questionnaire has been completed or expires.
    - **If you have limited internet access or are unable to access the link, please complete the Daily Travel Log on the paper form provided instead.**
    - If you are filling out the Daily Travel Log on the paper copies provided and are unable to complete a daily entry within 48 hours, please leave that day blank.
- On the day you return to the US, you will receive an additional email asking you to complete the **Post-Travel Questionnaire**.
    - You have 10 days to complete this questionnaire. You will receive an email reminder every 48 hours until the post-travel questionnaire has been completed or expires.
    - You will also need to **submit your second stool sample** and any paper Travel Log Forms that were completed in lieu of the web-based form. Materials to mail the stool sample and the questionnaires should have been provided to you at your pre-travel visit. ***If they were not recieved or if they were misplaced, please contact the travel clinic.***

- ***If you do not receive an email within 24 hours of returning from your trip, please contact the travel clinic.***

If your travel plans change or if you have any questions regarding the study, please contact The New York Center for Travel and Tropical Medicine:

Phone: +1 (646) 461-1125

E-mail: NYTravelStudy@gmail.com