IDCRP-115: A randomized controlled trial evaluating single dose rifaximin versus azithromycin with loperamide adjunct for treatment of acute watery diarrhea (TrEAT TD 2.0)

NCT05677282

Protocol
Date: 06/01/2026

EIRB Protocol Template (Version 1.15)

1.0 General Information

***Please enter the full title of your protocol:**

A randomized controlled trial evaluating single-dose rifaximin versus azithromycin with loperamide adjunct for treatment of acute watery diarrhea (TrEAT TD 2.0)

***Please enter the Protocol Number you would like to use to reference the protocol:**

IDCRP-115 (TrEAT TD 2.0); Version 8.0 (eIRB v1.15), 01 June 2026
* This field allows you to enter an abbreviated version of the Protocol Title to quickly identify this protocol.

Is this a multi-site protocol (i.e. Each site has their own Principal Investigator)?

No

Does this protocol involve the use of animals?

○ Yes ◉ No

2.0 Add sites

2.1 List sites associated with this study:

Is Primary?	Site Name
◉	**R and E** - Uniformed Services University of the Health Sciences (USUHS)

3.0 ■ Assign project personnel access to the project

3.1 * Please add a Principal Investigator for the study:

Name	Role	Training Record
Tribble, David R, MD DrPH	**Principal Investigator**	**View Training Record**

Responsibility

☐ Student
☐ Resident
☐ Site Chair
☐ Fellow

3.2 If applicable, please select the Research Staff personnel:

A) Additional Investigators

Name	Role	Training Record
[redacted]	Associate Investigator	View Training Record
[redacted]	Associate Investigator	View Training Record
[redacted]	Associate Investigator	View Training Record
[redacted]	Associate Investigator	View Training Record

B) Research Support Staff

Name	Role	Training Record
[redacted]	Team Member	View Training Record
[redacted]	Team Member	View Training Record
[redacted]	Team Member	View Training Record
[redacted]	Team Member	View Training Record

3.3 *Please add a Protocol Contact:

Name	Role	Training Record
[redacted]	Study Contact	View Training Record
[redacted]	Study Contact	View Training Record
[redacted]	Study Contact	View Training Record
[redacted]	Study Contact	View Training Record
[redacted]	Study Contact	View Training Record
[redacted]	Study Contact	View Training Record

The Protocol Contact(s) will receive all important system notifications along with the Principal Investigator. (i.e. The protocol contact(s) are typically either the Protocol Coordinator or the Principal Investigator themselves).

3.4 If applicable, please select the Designated Site Approval(s):

Name	Role	Training Record
No Designated Department Approval have been added		

Add the name of the individual authorized to approve and sign off on this protocol from your Site (e.g. the Site Chair).

4.0 Project Information

4.1 * What department(s) will be associated with this protocol?

Preventive Medicine

4.2 * Has another IRB/HRPP reviewed this study or will another IRB/HRPP be reviewing this study? If Yes, answer the questions according to the IRB/HRPP Determination.

○ Yes ◉ No

IRB Name	Review Date	Determination
: Other If other, please define: UK Ministry of Defence Research Ethics Committee (MODREC)		Approved via Full Board procedures Other Determination:
: Other If other, please define: Naval Medical Research Unit-EURAFCENT (Previously NAMRU-3)		Acknowledged Other Determination:
: Other If other, please define: Naval Medical Research Unit-SOUTH (Previously NAMRU-6)		Acknowledged Other Determination:
: Other If other, please define: Naval Medical Research Center		Not Human Subjects Research Other Determination:
: NMCP IRB		Not Human Subjects Research

If other, please define:		Other Determination:
: WRAIR IRB If other, please define: WRAIR-A	09/23/2024	Not Human Subjects Research Other Determination:
: Other If other, please define: USMC HRPP	01/30/2025	Administrative Review Other Determination: Concurrence
: WRAIR IRB If other, please define: WRAIR-AFRIMS	03/26/2025	Not Human Subjects Research Other Determination:

4.3 * Is this a research study or a Compassionate Use/Emergency Use/HUD project?

(•) Yes ( ) No

4.4 What type of research is this?

- [x] Biomedical Research
- [ ] Clinical trial (FDA regulated)
- [ ] Behavioral Research
- [ ] Educational Research
- [ ] Psychosocial Research
- [ ] Oral History
- [ ] Other

4.5 Are you conducting this project in pursuit of a personal degree?

( ) Yes (•) No

4.7 * Is this human subjects research? (As defined by 32 CFR 219) Human subject means a living individual about whom an investigator (whether professional or student) conducting research:
(i) Obtains information or biospecimens through intervention or interaction with the individual, and uses, studies, or analyzes the information or biospecimens; or
(ii) Obtains, uses, studies, analyzes or generates identifiable private information or identifiable biospecimens.

◉ Yes ○ No

4.8 * Do you believe this human subjects research is exempt from IRB review?

○ Yes ◉ No

5.0 Personnel Details

5.1 List any Research Team members without EIRB access that are not previously entered in the protocol:

Name: (Last, First, M.I.)	Phone Number:	Email Address:	Associated Institution:
[redacted] Role on Protocol: Biostatistician	[redacted]	[redacted]	USU
Name: (Last, First, M.I.) [redacted] Role on Protocol: Associate Investigator	Phone Number:	Email Address: [redacted]	Associated Institution: Naval Medical Leader and Professional Development Command
Name: (Last, First, M.I.) [redacted] Role on Protocol: Quality Assurance Specialist	Phone Number:	Email Address: [redacted]	Associated Institution: USU
Name: (Last, First, M.I.) [redacted] Role on Protocol: Associate Investigator	Phone Number:	Email Address: [redacted]	Associated Institution:
Name:			

(Last, First, M.I.) [redacted] Role on Protocol: Associate Investigator	Phone Number:	Email Address: [redacted]	Associated Institution: NMCP
Name: (Last, First, M.I.) [redacted] Role on Protocol: Associate Investigator (Balikatan Site PI)	Phone Number: [redacted]	Email Address: [redacted]	Associated Institution:
Name: (Last, First, M.I.) [redacted] Role on Protocol: Associate Investigator (CLDJ Site PI)	Phone Number:	Email Address: [redacted]	Associated Institution: U.S. Naval Medical Research Unit EURAFCENT
Name: (Last, First, M.I.) [redacted] Role on Protocol: Associate Investigator (SCAB Site PI)	Phone Number: [redacted]	Email Address: [redacted]	Associated Institution: U.S. Naval Medical Research Unit SOUTH

5.2 **Will you have a Research Monitor for this study?**

- (•) Yes
- ( ) No
- ( ) N/A

Research Monitor Qualifications
Ensure the individual has expertise consistent with the nature of risk(s) identified within your study and is independent of the team conducting the research.

Research Monitor Role:

Accruing safety data will be closely monitored by 2 safety review groups: one internal to the study team and the DSMB. The internal group includes the study PI and US and UK Site PIs. The DSMB composition is described above. The DSMB central research monitors will assume the role of site medical safety monitor and will be a physician (or other licensed clinician) with relevant expertise whose primary responsibility is to provide independent safety monitoring in a timely fashion. Participation will last for the duration of the study. The research monitors will have no direct involvement in the conduct of the study, and no financial, proprietary, professional, or other interests that may affect impartial, independent decision-making.

The primary focus of the central research monitors is to independently review all adverse events considered serious and unexpected. Clinical and laboratory data, clinical records, and other study-related records will be made available for review. The PI will ensure that the research monitors are apprised of all new safety information relevant to the study product and the study.

If applicable, you may nominate an individual to serve as the Research Monitor:

No Users have been selected.

6.0 Data/Specimens

6.1 Does the study involve the use of existing data or specimens only (no interaction with human subjects)?

○ Yes ◉ No

7.0 Funding and Disclosures

7.1 Source of Funding:

Funding Source	Funding Type	Amount
: Other Defense Health Program		1495680

Total amount of funding:

1495680

7.2 Do you or any other Investigator(s) have a disclosure of a personal interest or financial nature significant with sponsor(s), product(s), instrument(s) and/or company(ies) involved in this study?

○ Yes ◉ No

If Yes, complete and attach Conflict of Interest forms for all key personnel

8.0 Study Locations

8.1 Is this a collaborative or multi-site study? (e.g., are there any other institutions involved?)

◉ Yes ○ No

8.2 Study Facilities and Locations:

Institution	Site Name	Site Role	FWA or DoD Assurance Number	Assurance Expiration Date	Is there an agreement?	IRB Reviewing for Site
Navy	Camp Lemonnier, Djibouti	Performance site	FWA00014267	08/04/2030		: USUHS IRB #1
Army	British Army Training Unit Kenya	Performance site	FWA00017554	06/14/2027	: CRADA	: Other
	Soto Cano Air Base, Honduras	Performance site	FWA00010031	08/22/2029		: USUHS IRB #1
Navy	Naval Medical Research Unit-EURAFCENT	Laboratory analyses	FWA00014267	08/04/2030	: IAIR	: USUHS IRB #1
Navy	Naval Medical Research Unit-SOUTH	Laboratory analyses	FWA00010031	08/22/2029		: USUHS IRB #1
Navy	Naval Medical Research Center	Data analysis	FWA00000152	12/20/2029		: USUHS IRB #1
Navy	Naval Medical Center Portsmouth	Laboratory analyses	FWA00006001	08/08/2030		: USUHS IRB #1
Army	Walter Reed Army Institute of Research	Laboratory analyses	FWA00000015	09/09/2030	: CRADA	: WRAIR IRB
Army	Walter Reed Army Institute of Research-Philippines-AFRIMS Virology Research Unit	Laboratory analyses	FWA00000015	09/09/2030	: CRADA	: WRAIR IRB

	Fort Magsaysay	Performance site	FWA00000015	09/09/2030		: WRAIR IRB

Other:

Other Institution Site	Site Role	FWA or DoD Assurance Number	FWA or DoD Expiration Date	Is there an agreement?	IRB Reviewing for Site
No results found					

8.3 Are there international sites?

Attach international approval documents, if applicable, when prompted. Note: Ensure local research context has been considered

(•) Yes ( ) No

8.4 Is this an OCONUS (Outside Continental United States) study?

(•) Yes ( ) No

Select the area of responsibility:

Have you obtained permission from that area of responsibility? (This is a requirement prior to study approval)

(•) Yes ( ) No

9.0 Study Details

9.1 Key Words:

Provide up to 5 key words that identify the broad topic(s) of your study

9.2 Background and Significance:

Include a literature review that describes in detail the rationale for conducting the study. Include descriptions of any preliminary studies and findings that led to the development of the protocol. The background section should clearly support the choice of study variables and explain the basis for the research questions and/or study hypotheses. This section establishes the relevance of the study and explains the applicability of its findings

Travelers' Diarrhea (TD) Epidemiology and Effects on Deployed Forces

TD is a common problem during overseas travel, with an approximate incidence of 30-40% for a 2-week trip to a high-risk destination.[1] TD also remains a highly prevalent disease in the deployment setting. Forty-five percent of US military personnel serving in support of operations in Iraq and Afghanistan experienced diarrhea and reported decreased job performance for a median of 3 days.[2] Similarly high attack rates have been reported by the UK military, with up to a 60% attack rate during a 6-week military exercise in Kenya,[3] resulting in significant short-term morbidity. It is estimated that one complete duty day is lost per episode of TD, creating transient critical shortages that impact mission readiness, while increasing health care utilization and

adding to deployment-related costs.[4] Given that the estimated daily cost of keeping a troop member in a deployed setting is over $2,000, a cost-effective strategy for effective early treatment could significantly mitigate the impact of TD on military operations.[5]

Current Management of Travelers' Diarrhea

TD continues to be a major health concern in deploying troops and combat settings. To address these issues, evidence-based guidelines were published in 2017 for the treatment of TD.[5,6] These guidelines took a more functional approach to the grading of diarrhea severity and deviated from the traditional number of unformed stools passed in a 24-hour period. For example, an individual may have debilitating diarrhea due to symptoms such as fever and abdominal cramping but with few stools; however, on the other hand an individual may have frequent watery stools without many systemic or debilitating symptoms. By assessing the functional impact, diarrhea is more appropriately characterized as "mild (acute)" when the diarrhea is tolerable and not distressing and does not interfere with planned activities, "moderate (acute)" when the diarrhea is distressing or interferes with planned activities, "severe (acute)" when the diarrhea is incapacitating or completely prevents planned activities, or "persistent" when the diarrhea has lasted longer than 2 weeks. Under these definitions, recommendations for the treatment of diarrhea were based on the current literature and expert opinion and are the current, mainstream practice in the community. The updated TD clinical practice guidelines (CPGs) also emphasize the importance of antibiotic stewardship. Prior to this trial, there have been no civilian or military TD randomized controlled trials (RCTs) that have utilized restricted eligibility with all prior trials allowing for milder TD cases to be included. The experience that will be gained through the systematic application of the CPG recommendations will, in addition to the primary efficacy objective, provide critical information on implementation of the guidelines.

Based on the current guidelines, mild diarrhea that is tolerable and not distressing is usually treated symptomatically and without the use of antibiotics. Given that most diarrhea is self-limiting, it is felt that if the symptoms are mild that good hydration and the occasional use of loperamide or bismuth subsalicyclates to control the symptoms should be sufficient and will avoid any potential adverse effects secondary to the use of antibiotics such as the development of resistant bacteria, *Clostridium difficile* infection, or other more drug-specific side effects. However, antibiotics are recommended for moderate or severe diarrhea where there is functional limitation on the individual's activities. Current DoD Deployment Health Guidelines-recommended treatments for acute watery diarrhea are azithromycin 500 mg orally as a single dose with loperamide as the **first-line agent**, with levofloxacin 500 mg orally as a single dose with loperamide as an alternative agent (except in regions of the world where there is resistance).[5]

Further support for single-dosed regimens with loperamide were published as the "Trial Evaluating Ambulatory Therapy of Travelers' Diarrhea (TrEAT TD)" study. This trial looked at the treatment of acute watery diarrhea in the deployed setting with either single-dose azithromycin at 500 mg, levofloxacin 500 mg, or rifaximin 1650 mg, all paired with loperamide.[7] Rifaximin is a rifamycin derivative that is mostly a gut luminal agent with very little systemic absorption and is currently FDA approved for the treatment of hepatic encephalopathy, irritable bowel syndrome without constipation, and TD. Given that 96.6% of the drug is excreted unchanged in the feces, it has very little systemic interaction with other drugs and has a very low side effect profile when used to treat travelers' diarrhea. In addition, significant fecal concentrations of 8000µg/g are achieved in the stool after a three-day course of 800 mg per day, which may be prolonged with use of gut anti-motility agents.[14] Clinical cure measured by resolution of diarrhea and return to daily activities at 24 hours was similar between the three arms and was achieved by 81.4%, 78.3%, and 74.8% of participants receiving levofloxacin, azithromycin, and rifaximin, respectfully. Treatment failures were uncommon and were only 3.8%, 4.4%, and 1.9% for participants receiving levofloxacin, azithromycin, and rifaximin, respectfully. Although azithromycin was found non-inferior to levofloxacin, non-inferiority could not be shown for rifaximin at 24 hours but was equivalent at 48 and 72 hours with cure rates of 90.8% at 48 hours and 96.0% at 72 hours. The use of high dose rifaximin as a single dose therapy was a proof of concept but was not intended to be the final dose regimen given the high cost. The TrEAT TD findings continue to support single-dose antibiotic therapy with loperamide. Given that this trial will limit eligibility to patients with functional impairment at time of initial clinic presentation, it is important to evaluate 24-hour cure rates (used as the primary outcome in the TrEAT TD study) in the subset with some limitation on activities due to diarrhea severity. There were approximately 25% of TrEAT TD participants who reported no impact on activity level /function. Focusing on the two regimens being compared in the upcoming trial, azithromycin 24-hour cure rate was 75.6% and rifaximin was similar at 72.4% in participants with functional impairment. As the eligibility criteria becomes more stringent with more severe TD cases the expectation for clinical recovery cannot reasonably be expected to be as short as 24-hours in a substantial proportion of participants. The primary endpoint to assess the non-inferiority of rifaximin compared against azithromycin will assess the time required to reach the last unformed stool that meets the diarrhea definition. Secondary outcomes will evaluate the resolution of non-

diarrhea associated symptoms as well as potential for relapse after 72-hours to assure a comprehensive assessment.

The TrEAT TD study demonstrated 'proof of concept' that the nonabsorbable antibiotic, rifaximin, has efficacy when delivered as a single high dose (1650 mg) with loperamide, which slows the fecal excretion of the drug. The high dose selected for TrEAT TD was used to assure an optimal test but was not meant to be a regimen to recommend for clinical use given the high cost. The DoD Deployment-related diarrhea CPG did not include single dose rifaximin as a treatment option but does refer to multi-day multi-dose rifaximin regimens that have an FDA license for use. Single dose antibiotic regimens with loperamide, which can be administered as direct observed treatment by deployed providers, are a much more practical and effective means to assure adherence and prompt return to duty. **This trial will determine if single dose rifaximin as a single 550 mg pill with loperamide should or should not be included as a recommendation in an update of the CPG.** If shown to be noninferior to the standard of care, azithromycin plus loperamide, then rifaximin which has a superior safety profile to azithromycin, theoretic advantages on less TD treatment-associated dysbiosis and antimicrobial resistance (AMR) emergence, plus importantly reserving azithromycin for TD rescue therapy while limiting overuse of this critical antibiotic used in deployment medical care across a wide range of infectious syndromes.

Potential Advantages of Rifaximin

Possibly Less Antimicrobial Resistance Concerns
The intestinal tract is an important reservoir for antibiotic-resistant organisms. Fecal acquisition of ESBL-PE during travel is independently associated with TD, self-treatment with antibiotics and loperamide, and visiting high ESBL-PE prevalence destinations (e.g., South and Southeast Asia).[8] More recent studies have shown that use of antibiotics for travelers' diarrhea increase the risk of colonization with an ESBL-PE but are usually not associated with any symptoms in the average healthy traveler. The population health consequences in those patients that are carriers of ESBL-PE affected by trauma or illness is still unknown. The proposed therapy could potentially have an advantage in not selecting for these resistant organisms given its shortened course, lower dose, and possibly beneficial effects on the gut microbiome.

Potentially Beneficial Effect on Post-Infectious Functional Gastrointestinal Disorders (FGID)
Pathogen-mediated disruption of the gut mucosal barrier, and persistent immune activation and dysbiosis have also been implicated in the development of long-term TD sequelae such as functional gastrointestinal disorders (FGID).[9] Symptoms of irritable bowel syndrome (IBS), based on the Rome criteria, can occur in 5-10% of TD cases.[10] Early treatment may reduce the risk of this sequelae given that those with more severe and more prolonged diarrhea are at higher risk for post-infectious IBS. This has not been shown clinically in humans and is one of the exploratory aims of this study; to see if the type of antibiotic and severity of illness have a similar effect on functional bowel disorders.

In this arena, rifaximin may have an advantage given that there is evidence that it has less of an impact on the microbiota than other antibiotics. Although it has a broad spectrum of activity, to include Gram-positive, Gram-negative, aerobic, and anaerobic bacteria, it seems to have little effect on the intestinal microflora. Even after two weeks of rifaximin, participants only experienced a 1 log reduction in intestinal coliforms per gram of stool. It also has activity against *C. difficile* and is a second-line treatment for *C. difficile* colitis meaning that this usual antibiotic-associated issue is unlikely with this drug.[11] For this reason, it has been used as an effective treatment in both small intestinal bowel overgrowth (SIBO) and IBS and it may be more beneficial in preventing functional bowel disorders due to infectious diarrhea that disrupt the normal bowel flora.

9.3 Objectives/Specific Aims/Research Questions:

Describe the purpose and objective(s) of the study, specific aims, and/or research questions /hypotheses

Primary:
To assess the clinical efficacy of rifaximin at a low dose (550 mg) with loperamide in treating acute watery diarrhea among ambulatory participants as compared to the current recommended

treatment for acute watery diarrhea with azithromycin 500 mg with loperamide. The primary efficacy endpoint is the time to last unformed stool (TLUS). TLUS is calculated from the time of antibiotic dose to last unformed (diarrheal) stool (TLUS) that meets the TD definition.
Secondary:

- Additional measures of clinical efficacy will be evaluated including cure rates at 24h, 48h, and 72h (this will consider if the TLUS has occurred within the respective 24h period as well as resolution of all TD-associated symptoms reported as moderate or greater); TD recurrence by the 7d follow-up following initial resolution.
- Assess safety and tolerability of treatment regimens.

Exploratory:

- Evaluate the differences in risk between the two treatment regimens for post-infectious functional bowel disorders.
- Evaluate the recovery of extended-spectrum beta-lactamase (ESBL)-producing Enterobacteriaceae post-treatment.
- Evaluate the rifaximin susceptibility in clinical isolates as available.
- Evaluate the microbiome changes in participants prior to treatment and at time-points after treatment to evaluate pathogen-specific and/or antibiotic treatment effects.
- The following exploratory laboratory objectives are contingent on identification of additional funding:
    - Evaluate the proteomic changes in participants using serum between acute and convalescent time-points to evaluate pathogen-specific and/or antibiotic treatment effects.
    - Evaluate serologic responses to selected enteropathogens compared to standard microbiological work-up for enteropathogens.

9.4 Study Design:

Describe study design in one to two sentences (e.g., prospective, use of existing records/data /specimens, observational, cross-sectional, interventional, randomized, placebo-controlled, cohort, etc.). Specify the phase – Phase I, II, III, or IV – for FDA-regulated investigational drug research

This study is a randomized, double-blind, active-controlled, treatment trial of TD in an outpatient setting enrolling deployed United States (US) to JTF-Bravo, Soto Cano Air Base, Honduras; Camp Lemonnier, Djibouti (CLDJ); and Exercise Balikatan 2025 and United Kingdom (UK) military personnel to British Army Training Unit, Kenya (BATUK). Enrollment is targeted to be completed within 3 years.

9.5 Target Population:

Describe the population to whom the study findings will be generalized

Active duty military personnel deployed to Kenya (UK military) or Honduras or Djibouti or the Philippines (US military) ages 18-60 years presenting with acute watery diarrhea who are deemed suitable for ambulatory therapy. Children and others who cannot provide informed consent will not be included in the trial.

9.6 Benefit to the DoD:

State how this study will impact or be of benefit to the Department of Defense

Deployment-associated traveler's diarrhea remains one of the most common medical ailments impacting personnel and continues to limit readiness intermittently in deployed forces. In addition to prevention practices to assure safe food and water, a critical means to mitigate the impact on force readiness is prompt and effective treatment. Single dose antibiotic regimens with loperamide, which can be administered as direct observed treatment by deployed providers, are a much more practical and effective means to assure adherence and prompt return to duty. **This trial will determine if single dose rifaximin as a single 550 mg pill with loperamide should or should not be included as a recommendation in an update of the CPG.** If shown to be noninferior to the standard of care, azithromycin plus loperamide, then rifaximin

which has a superior safety profile to azithromycin, theoretic advantages on less TD treatment-associated dysbiosis and AMR emergence, and possibly plus importantly reserving azithromycin for TD rescue therapy while limiting overuse of this critical antibiotic used in deployment medical care across a wide range of infectious syndromes.

10.0 Study Procedures, Data Management, and Privacy

10.1 Study Procedures:

Describe step-by-step how the study will be conducted from beginning to end

A Cooperative Research and Development Agreement (CRADA) has been executed between USU, HJF, and UK MOD to cover the roles and responsibilities related to the research as well as the sharing of data and specimens.

The UK will have a separate protocol that is reviewed and approved by the UK Ministry of Defence Research Ethics Committee (MODREC). The USU IRB is the IRB of record for the US DoD sites (USU, Soto Cano AFB, NAMRU-SOUTH, NAMRU-EURAFCENT, CLDJ, Fort Magsaysay [Exercise Balikatan], Air Force Research Institute of Medicine [AFRIMS] and NMRC). Since the study is funded by US DoD, DoD Human Research Protection Official (HRPO) concurrence for the UK protocol was obtained prior to initiation.

Description of Protocol Drugs & Dosage Justification
All study drugs will be purchased through a contracted research pharmacy and stored per manufacture specifications.

Loperamide is dispensed as a 2 mg capsule to be taken orally. For this study, it will be provided as 4 mg (2 capsules) initially, to be co-administered with blinded antibiotic, followed by 2 mg (1 capsule) after each loose stool, up to 16 mg (8 capsules) per day. Loperamide is an over-the-counter drug, but the dosing is limited to 16 mg / day over the counter. The number of loperamide capsules used by a participant following initial administration will be recorded.

Azithromycin is a macrolide and has been licensed by the FDA for human use. Licensed indications for which it has been approved include pulmonary infections, sinusitis, skin infections and genitourinary infections. [Package Insert: www.pfizer.com] It has been extensively evaluated in several clinical trials and been found to be effective in the treatment of TD caused by a number of bacterial pathogens such as diarrheagenic *E. coli*, *Shigella* spp. and *Campylobacte r* spp. It is the preferred antibiotic for the empiric treatment of travelers' diarrhea. Azithromycin is safe when administered in doses as high as 1,200 mg. For the purposes of this trial, we will use a one-time 500 mg dose in conjunction with loperamide for the treatment of acute watery diarrhea per current treatment guidelines.

Rifaximin, a rifampin derivative, is a gut-targeted, minimally absorbed, broad-spectrum, oral antibiotic that has been licensed for treatment of TD and has been studied extensively for the treatment of other gastrointestinal (GI)-based conditions such as hepatic encephalopathy (HE), IBS, and *Clostridium difficile* infection. Rifaximin (XIFAXAN®, Bausch Health Companies, Inc., Laval, Canada) is approved in the US for the treatment of TD caused by non-invasive strains of *E. coli* in patients 12 years of age or older at a dosage of 200 mg 3 times daily (TID) for 3 days (total dosage 1800 mg). Rifaximin is also approved for the treatment of HE (550 mg twice daily) and for IBS with diarrhea (550 mg, TID for 14 days). Rifaximin has also been studied in the US for the treatment of conditions including TD prophylaxis (600 mg & 1100 mg once daily for 14 days), *C. difficile*-associated diarrhea (400 mg TID for 10 days), HE (doses up to 2400 mg/ day), and travelers' diarrhea (1650 mg single dose). No increases in side-effects have been seen compared to placebo in these prior studies. This study will evaluate a 550 mg single-dose course of rifaximin which should provide adequate prolonged fecal concentrations that are as effective as multi-dose regimens (200 mg TID for 3 days) or a single, high-dose regimen (1650 mg, single dose), based on previously described pharmacodynamics.[14]

Concomitant medications: There have been no significant drug-drug interactions reported with loperamide, rifaximin, or azithromycin. Co-administration with magnesium- or aluminum-containing anti-acids or ferrous sulfate does not reduce the bioavailability of azithromycin or the luminal concentrations of rifaximin. Therefore, patients will not need to separate the ingestion of any anti-acid medication in relation to when they take the study drug. While no interactions have been noted with theophylline, digoxin, or warfarin, cautious clinical practice dictates close monitoring of drug level or international normalized ratio (INR) during co-administration. Given

our inability to adequately monitor levels in the field, participants who are currently taking any of these 3 medicines will be excluded from the study.

Interventional Groups & Study Drug Administration
Participants will be randomized to receive one of 2 regimens:

1. 75 participants – single-dose rifaximin (550 mg) plus loperamide (4 mg); or
2. 75 participants – single-dose azithromycin (500 mg) plus loperamide (4 mg)

In addition to the study drugs detailed above, participants will be instructed to take 2 mg (1 capsule/tablet) of loperamide after each loose stool (not to exceed 16 mg/day for 2 days). Participants will be provided 6 additional 2 mg capsules/tablets of loperamide at enrollment and directed to take them as described. Eight additional 2 mg capsules/tablets of loperamide will be provided to participants during the 24 hour follow up visit as needed.

Randomization & Blinding Procedures
Study drugs will be procured by a contract research organization for opaque packaging and labeling so the specific drugs cannot be ascertained to assure blinded treatment assignment. The following is the CRO Pharmacy product preparation (done similarly for azithromycin and rifaximin):

Preparation of Study Drugs per 4 capsules

1. A batch number will be assigned, batch labels will be prepared.
2. One Study Drug tablet (rifaximin 550 mg or azithromycin 500 mg) tablet and four empty capsule shells will be counted.
3. Empty DB capsules will be placed into ProFill capsule filling system to separate caps from the bodies of the capsules.
4. Each Study Drug tablet will be cut in approximately half using a tablet cutter. Each half tablet will be cut to approximately a quarter of a tablet using a tablet cutter.
5. Each ¼ of a tablet will be placed into an empty body of a capsule while it is in a Profill capsule filling system. Microcrystalline cellulose will be added to the body of the capsule containing ¼ of Study Drug tablet using spatula.
6. Filled capsules will be taken out of the machine and cap will be placed on the body of a filled capsule by hand.
7. Caps and bodies will be locked together and the capsules will be cleaned.
8. Four capsules plus two 2 mg tablets of loperamide will be heat-sealed in a mylar bag and labeled with a study label including batch number, Study Number and Treatment Number as per the randomization schedule provided.
9. Steps 2-8 will be repeated for each Study Drug dose.
10. Production information will be documented on the Compound Form.

Allocation of treatment will be based on a blocked randomization by site. The individually packaged treatment packs will be labeled with the study identification number and Treatment Number as per the randomization schedule.

The randomization schedule and codes for both study arms will be provided by the IDCRP biostatistician for each treatment assignment. In the event of a medical emergency (such as serious medication-related allergic and/or adverse reaction or disenrollment due to hospitalizing participant due to disease progression) it may be necessary to break the double-blind code for that individual.

Each of the medicines is heat stable and can be maintained at room temperature during the study. Unused doses of the medication will be destroyed at the completion of the study.

Participants meeting the inclusion criteria will be assigned a sequential "Treatment Number" linked to a randomized treatment based on a block randomization. The study physician will dispense the study medication (labeled with the appropriate "Treatment Number") to the participant. A cup of water will be made available to the study participant to swallow the assigned study drugs. The participant will be observed for a 30-minute period to monitor for immediate adverse reactions.

Study Definitions

The primary endpoint is **Time to Last Unformed Stool (TLUS)**.
Time to Last Unformed Stool (TLUS) = calculated as the time from taking the first dose of study medication until passage of the last unformed stool that continues to meet the diarrhea definition

(stated below). The TLUS requires a full ascertainment of the diarrhea episode in order to determine the last diarrheal stool that fell within a 24h block when the participant continued to meet the TD definition.

TD definition – a participant meets the definition by either having 3 or more grade 3-5 stool in a 24h period OR 2 grade 3-5 stools in 24 hours plus diarrhea-associated symptoms (listed below) in the same 24-hour block. To be clear, meeting the TD definition during follow-up is NOT the same as the eligibility criteria which also requires the participant to meet a level of severity limiting activities. TD definition in follow-up is used to determine the TLUS as the participant is clinically improving.

The stool characteristics are based on the following:

Grade 1: Fully formed (normal)
Grade 2: Soft (normal)
Grade 3: Thick liquid (diarrheal)
Grade 4: Opaque watery (diarrheal)
Grade 5: Rice-water (diarrheal)

Fever = oral temperature >100.4º F (38.1º C)
Dysentery = visualized gross blood in diarrheal stool (grade 3-5) confirmed by (+) hemoccult test
Diarrhea-associated signs/symptoms = abdominal pain or cramps, nausea, vomiting, fever, tenesmus, and gross blood in stools temporally related to the diarrheal episode
Figure 1. Study flow diagram

Enrollment
See sections on Recruitment and Consenting for specific details regarding those activities. Information about the participant's past medical history, details of the current illness, and risk factors for diarrhea will be collected using a standardized questionnaire followed by having a standard physical exam performed. A stool specimen (if not already provided) and a 10 mL sample of blood will be collected. Female participants will also be required to submit a urine sample for pregnancy testing. A portion of the collected stool sample will be smeared onto a Whatman® FTA® Elute Micro Card and put in a OMNIgene GUT tube by study staff at the clinical sites. If a site does not have the capacity to process and store stool and/or serum, the remaining stool will be discarded, and the serum will not be collected.

Participants will be randomized to a treatment and will be provided the single-dose antibiotic and initial dose of loperamide. Participants will be observed for 30-minute following antibiotic dose to assess for treatment related adverse reactions. Participants will be instructed on the use of additional loperamide for continued loose stool. The research staff will provide the participant with the study diary and will provide instruction for completion. Participants will also be directed by the research staff to return to clinic and/or contact the study investigator on site if illness is becoming more severe.

Clinical Monitoring
Participants will be asked to follow-up at 24 hours (+/- 6 hours), 72 hours (+/- 12 hours), and 7 days (+/- 2 days) after enrollment to perform a clinical assessment (and may include an interview regarding symptoms associated with illness, a physical exam, collection of vital signs, and repeated pregnancy testing for females of childbearing potential where the principal investigator or designee is uncertain of the pregnancy status). Participants will be given diaries to complete daily. Information to be included on the diary includes time of loperamide usage, the number and form of all stools passed as well as the presence of enteric symptoms, disability, and global wellness assessments. The diary cards, which will act as memory aids, will be reviewed by the investigator with the participant during each follow-up clinic visit until wellness is achieved, for a minimum of 3 days. Additional follow-up at 21 days (- 7/+14 days) will be conducted to monitor changes in bowel flora. Use of concomitant medications and occurrence of adverse events will be assessed at all follow-up visits. Safety will be assessed at each clinic visit. The primary focus of safety assessment is in the first 3 days. In addition to the priority time period to monitor safety during the 3 days after antibiotic dose, safety assessments will also be done at the Day 7 and 21 study visits to assess for illness recurrence as well as capture any unsolicited new onset symptoms as well as concomitant prescription medications.

Virtual follow-up visits with self-collected sampling may be utilized if an in-person follow-up visit is not feasible within the study windows for the Day 7 or Day 21 visits. Participants with virtual follow-up for the Day 21 visit will be contacted by study staff for safety assessment and to capture any changes in concomitant medications.

Stopping Criteria

The following guidelines will be used to stop the study and trigger interim safety analysis, although any participant already administered study product will continue to be followed per protocol. The following stopping rules apply to events associated with treatment, when attributed to administration of antibiotics and/or loperamide by the investigators/safety review group.

1. The development of toxic megacolon or any serious adverse event which is considered a related outcome to the use of the antibiotic or combination treatments for TD
2. Participant develops a SUSAR which is considered a related or suspected to be related outcome to the use of the antibiotic or combination treatments for TD

24- and 72-hour follow-ups

The follow-up evaluations at 24 and 72 hours are designed to measure both disease progression /resolution and potential drug toxicity. The clinical assessment at 24 hours will include a general medical assessment and a determination as to whether further therapy other than loperamide is necessary. If symptoms have improved or have not significantly worsened, participants will be advised to continue loperamide as prescribed, but no changes to the antibiotic treatment regimen will be made. If the patient's symptoms have significantly worsened (e.g., development of fever, bloody stools, dehydration requiring intravenous repletion, change in clinical condition requiring hospital admission, or any other situation in which the study physician feels additional treatment modalities are needed), the participant may be put on a rescue therapy and/or treatment code may be broken, and the participant will be treated according to the clinician's judgment. Participants will be asked to give an overall assessment of how well they are feeling from the diarrheal illness at the 24- and 72-hour follow-up visits. At the 24-hour follow-up visit, a standardized questionnaire will be completed by participants to assess existence of functional bowel disorders in the previous 3 months.

Participants' daily diaries will be evaluated and collected after the 72-hour and Day 7 (if necessary) follow-ups.

It is anticipated that a minority of participants will fail treatment during the 72-hour clinical monitoring period when the primary endpoint for efficacy is being evaluated. In the event this occurs, the study physicians will first determine if the participant's illness continues to require antibiotic treatment. Traveler's diarrhea is a self-limited illness and, as emphasized in the eligibility determination, it is important to determine if antibiotics are still required. Oftentimes, the illness may not have resolved fully but additional antibiotics are not warranted because the illness now falls into the mild severity category and is not impacting the participant's functional status. In contrast, if the participant's illness is worsening or continues to impact functional capacity (as described in the eligibility determination) than antibiotic treatment is warranted. In these cases, the participant's treatment assignment needs to be known (unblinding for only this specific participant) so the study physician can appropriately prescribe rescue therapy with a different antibiotic. These clinical failures are expected to occur and are not a basis for halting the study.

7-day follow-up

The Day 7 follow-up visit may be conducted as an in-person or virtual follow-up visit.

At an in-person 7-day follow-up visit, participants will be asked to provide a second stool specimen and a second 10 mL blood specimen. A portion of the collected stool sample will be smeared onto a Whatman® FTA® Elute Micro Card and placed in an OMNIgene GUT tube by study staff at the clinical sites. If a site does not have the capacity to process and store stool and /or serum, the remaining stool will be discarded, and the serum will not be collected. Participants have a clinical assessment similar to those at the 24- and 72-hour follow-ups. Should a UK enrollee be returning to the UK after the 72-hour visit but prior to the Day 7 visit, he/she may be seen for the Day 7 follow-up in the UK.

For a virtual Day 7 visit, participants will self-collect a stool sample using the OMNIgene kit and a stool card as well as self-collect a 40 µL blood sample via finger prick using the Neoteryx Mitra® microsampling kit. The Mitra sample dries on the collection tip. Information regarding the Mitra collection kits is available at URL: **https://www.neoteryx.com/home-blood-blood-collection-kits-dried-capillary-blood** (last accessed: 26 May 2023).

21-day follow-up

The Day 21 follow-up visit may be conducted as an in-person or virtual follow-up visit.

For an in-person Day 21 visit, participants will return on Day 21 (up to day 35) to provide a stool specimen and a 10 mL blood specimen. A portion of the collected stool sample will be smeared onto a Whatman® FTA® Elute Micro Card and placed in a OMNIgene GUT tube by study staff at the clinical sites. If a site does not have the capacity to process and store stool and/or serum,

the remaining stool will be discarded, and the serum will not be collected. If a US or UK enrollee returns to their home country prior to 21 days and can be followed up by study staff, a stool and blood specimen may be collected.

For a virtual Day 21 visit, participants will self-collect a stool sample using the OMNIgene kit and a stool card as well as self-collect a 40 µL blood sample via finger prick using the Neoteryx Mitra® microsampling kit.

Efforts should be made to perform a virtual Day 21 visit if an in-person visit is not feasible. This visit is not required to be considered for the primary outcome determination but is important for the study's secondary outcomes. If this visit is missed this will not constitute a protocol deviation.

Long-Term Follow-Up for Post-Infectious Functional Bowel Disorders
Participants will complete a baseline questionnaire regarding functional bowel disorders at the 24-hour visit and will be contacted via email or text message to complete a follow-up questionnaire at 3 months post-enrollment. If a participant does not complete the questionnaire after the first contact attempt, 4 subsequent attempts will be made, 1 attempt every 2 weeks for up to two months. The questionnaire will assess for several types of functional bowel disorders using standardized questions and definitions.

Additionally, for US participants, the Military Health System Data Repository (MDR) will be queried to identify diagnoses of functional bowel disorders and joint-related conditions that may represent post-infectious sequelae up to one year after study enrollment. The UK military investigators will review the UK National Health System (NHS) electronic medical records for the UK MOD enrolled participants to assess for similar post-infection diagnoses as done for US military participants.

Collection of the Human Biological Specimens
This study will prospectively collect blood and stool on Days 0, 7, and 21. Stool samples will be used for microbiological analysis to assess etiology of diarrhea as well as bacterial isolate antibiotic susceptibility. Serology will be utilized to attempt to determine seroconversion status for pathogens not identified during stool analysis. Specimens will be sent to the research labs for batched testing and are not used for clinical management. Allowance for analysis of exploratory laboratory objectives is included in a participant's consent into the study. Residual samples and isolates will be preserved for future analysis (also included in the participant informed consent).

Fecal Sample Collection & Processing
All stool specimens (Days 0, 7, and 21) will be either collected in stool cups or using a toilet accessory (self-collected samples for virtual visits). Study staff will be trained for specimen collection and storage. Refrigeration equipment and specimen transportation logistics from clinical study site to the site-associated sample processing lab will be in place. Details on frequency and method of transport of specimens from clinical study site to processing lab will be detailed in the Laboratory Manual of Procedures (LMOP). The collection of all stool samples will be documented by one of the study staff. All specimens will be labeled with their unique participant ID number and specimen number.

For in-person visits, one single stool sample will be collected in a plastic container. Portions of the specimen will be transferred into modified Cary-Blair transport medium or liquid (for bacterial determination) and the remainder of the stool sample in the initial plastic container will be divided into additional aliquots. Aliquots from residual stool specimens will be frozen at -70°C for real time PCR analysis for future testing. Required equipment at the clinical study site include a 4°C refrigerator, -70°C freezer, surge protectors, and power backup modules to store stools samples while awaiting transportation to the site-associated clinical laboratory.

Whatman® FTA® Elute cards collected using Day 0 (Enrollment Visit), Day 7, and Day 21 stool samples will be temporarily stored and shipped to the Naval Medical Center Portsmouth (NMCP) for testing using PCR (using the TACC enteropathogen TD panel as well as screening panel for AMR genes as detailed below).

OMNIgene GUT tubes collected using Day 0 (Enrollment Visit), Day 7, and Day 21 stool samples will be temporarily stored and shipped to the NMCP for long-term storage. Shipment for microbiome testing will be to the collaborators at Lawrence Livermore National Lan (LLNL). Additional storage and transportation details will be described in the LMOP.

Blood Specimen Collection
A 10 mL sample of blood will be collected for in-person visits on Days 0, 7 and 21. The samples will be collected in a red top tube and allowed to clot for 1 hour at room temperature. Samples

will then be centrifuged at 1500 RPM for 15 minutes, the serum decanted, placed in freezer tubes and stored at −70°C until transported to an IDCRP specimen repository pending shipment to a lab for serologic and/or immune proteomic testing.

For virtual visits (Day 7 and Day 21), a 40 µL self-collected blood sample will be collected using the Neoteryx Mitra® microsampling kit. The collected Mitra sample will either be returned to the site study staff for batch shipping to NMCP or shipped by the participant using a pre-paid mailer.

Pathogen Identification
The BioFire® FilmArray® Gastrointestinal (GI) Panel (Biomerieux) will be used as the initial diagnostic panel at clinical sites for bacterial, parasitic and viral enteropathogens. The BioFire® GI Panel is a FDA-cleared diagnostic that tests for 22 of the most common enteropathogens via real time PCR to include *Salmonella*, *Shigella*, *Campylobacter*, *Vibrio* the diarrheagenic *E.coli*, norovirus, *Giardia lamblia*, *Entamoeba histolytica*, and *Cryptosporidium*.

All stool samples will be plated that test positive for a bacterial pathogen from stool sample collected on Day 0 on selective and differential media for enteric bacterial pathogen isolations. Single colonies exhibiting the proper characteristics on the various media will be further processed using an automated bacterial identification platform for biochemical identification and antibiotic susceptibility testing based on the LMOP. Five (5) *E. coli* (non-O157:H7) isolates identified will be evaluated for key virulence factors that are often associated with enterotoxigenic 5 (ETEC) and enteroaggregative 5 (EAEC) by multiplex PCR.

Isolates that are identified as *Salmonella*, *Shigella*, *Campylobacter* or *Vibrio* spp. will be further characterized using monoclonal antibody-based enzyme immunoassays and/or by multiplex PCR. Antimicrobial testing will be performed on selected isolates. All identified bacterial isolates will be stored at -70°C in Tryptic Soy Broth (TSB) and 15% glycerol.

ESBL-producing Enterobacteriaceae will be detected using standard microbiological laboratory techniques. Both selective chromogenic agar and cefpodoxime disc diffusion testing will be used for faecal screening for detection of ESBL-producers. Any isolates identified by chromogenic agar and/or as being resistant to cefpodoxime will be subjected to ESBL confirmatory testing.

All suspected ESBL isolates will be stored at -70°C in TSB and 15% glycerol. Confirmatory testing will either be performed locally by rapid molecular testing or stored isolates will be analysed in the UK using combination disc testing or PCR.

Stool PCR Cards
The stool samples obtained from participants on Day 0, Day 7, and Day 21 will be smeared onto a Whatman® FTA® Elute Micro Card by study staff at the clinical sites for in-person visits or by the participant for virtual visits. Each stool card will be placed in a separate multi-barrier pouch and stored at room temperature. The stool cards will be mailed to the research laboratory at NMCP in batches or directly from participants using pre-paid mailers.

The stool PCR assay was developed at the University of Virginia and NMCP, as part of the TRAVMIL (IDCRP-037) and TrEAT TD studies. Testing for the following pathogens will be conducted using the multiplex PCR assay on stool cards: astrovirus, adenovirus, rotavirus, norovirus, sapovirus, *Aeromonas* spp., *Campylobacter* spp., *Campylobacter jejuni*, *Campylobacter coli*, *Clostridium difficile*, Yersinia enterocolitica, *Bacteroides fragilis*, *Salmonella enterica (S enteritidis, S paratyphi, S typhi and S typhimurium)*, *Vibrio parahaemolyticus*, *Shigella* spp., enteroinvasive *Escherichia coli* (EIEC) shiga-toxin producing *Escherichia coli (*STEC) including 0157:H7, enteroaggregative *Escherichia Coli* (EAEC), enteropathogenic *Escherichia coli* (EPEC), ETEC (heat-labile toxin [LT], heat-stable toxin[ST], and colonization factors), *Plesiomonas shigelloides*, *E. histolytica*, Cryptosporidium, *Cyclospora cayetanensis*, *Cystoisospora belli*, *Enceph alitozoon intestinalis*, *Enterocytozoon bieneusi* and *Giardia lamblia*.

An additional stool PCR assay developed by the University of Virginia and modified by NMCP will test for acquisition of antimicrobial resistance genes (AMR). This TAC contains over 70 genes or mutations associated with antimicrobial resistance (i.e. genes for β-lactamase production, fluoroquinolone, macrolide, aminoglycoside, tetracycline, TMP/SMX resistance). The AMR TAC tests for the following AMR classes: carbepenemase (NDM, VIM, KPC, LAT, IMP, SME, NMC, SPM), beta-lactamase (CTX-M, SHV, TEM, SFO, PER, VEB, OXA), ampC and other beta-lactamases (ACT, CMY, ADC, DHA, LEN, MAL, PAO, OKP, OXY), aminoglycoside resistance (aac, aadA, aadB, aph, strA,strB, armA, ant(6),rmt), fluoroquinolone resistance (gyrA, parC) and macrolides (ere,erm, mph, msr, 23S2075G). Samples smeared on the Whatman® FTA® Elute Micro Card at Day 0 and Day 21 will be tested for AMR genes.

Proposed Methodology for Exploratory Objectives (contingent on future funding); specific details of testing methods may vary from what is described below

OMNIgene®•GUT
OMNIgene®•GUT is an all-in-one system for easy self-collection and stabilization of microbial DNA from feces for gut microbiome profiling. It rapidly homogenizes and stabilizes at the point-of-collection and has the ability to store stabilized DNA at ambient temperature for 60 days.

16s rRNA and/or Other Sequencing for Microbiome Evaluation (performed at LLNL)
For exploratory analysis, participants may have microbiome evaluation completed on available frozen stools or OMNIgene®•GUT samples from Day 0, 7 and 21 with stool samples separated into 5 mL aliquots in cryovials and stored at -80°C. Frozen stool aliquots from the selected participants will undergo DNA extraction using a Qiagen QIAmp DNA Stool minikit according to manufacturer's instructions. Next-generation sequencing technology (Illumina HiSeq2500) will be used to sequence the V4 region of the 16S ribosomal RNA. In order to do this, a 286-base pair (bp) region will be amplified using F515 (Forward): GTGCCAGCMGCCGCGGTAA and R806 (Reverse): GGACTACHVGGGTWTCTAAT primers. The primers are tagged at their 5' end by unique 8 base pairs. PCRs will be conducted to amplify genomic material. The reactions will contain 5-100 ng DNA template, 0.5 µL KAPA DNA Polymerase (KAPA Biosystems), and 2 pmol of each primer. The reaction conditions will consist of an initial 95°C for 5 min followed by 30 cycles of 98°C for 20 sec, 65°C for 15 sec, and 72°C for 25 sec, and a final extension of 72°C for 5 min. Amplicons will be analyzed by gel electrophoresis and pooled prior to purification using the Qiagen kits according to manufacturers' instructions. Sequencing libraries will be prepared by Illumina TruSeq DNA library prep kits per manufacturers' instructions. Sequencing libraries will be quantified using qPCR using KAPA reagents (KAPA Biosystems) and sequencing data of 151 bp paired ends (PE) will be generated on Illumina's HiSeq 2500 instrument.

The human gut microbiome is comprised of a vast array of microbes including bacteria, fungi, archaea and viruses. The composition and stability of the microbiome community has been associated with human health and disease, both chronic GI conditions such as IBD, as well as acute susceptibility to infectious diarrhoea. The virome includes eukaryotic viruses and bacteriophage viruses, both emerging as influencers upon host health. Identification of these viruses is still underway, we intend to test stool samples for both eukaryotic and bacteriophage viruses with the intention of advancing knowledge of these microbes, specifically in the geographical locations of military deployments. By understanding the host virome on Operations we may be better prepared to preserve GI health and enhance Operational capability.

Upon collection fresh stool will be frozen, stored and shipped at -80 degrees. Virus like particles (VLP) will undergo enrichment and the filtrates treated to degrade any unfiltered bacterial and host cell membranes. The VLPs will then be lysed and nucleic acid extracted. VLP DNA will undergo amplification then sequencing on an Illumina MiSeq instrument. They will then be assigned a taxonomy. Virome analysis will be coordinated and completed through the UK MOD collaboration.

Microbiome testing and antimicrobial resistance gene testing for exploratory endpoints
Metagenome shotgun sequencing may be performed on pre- and post-treatment stool specimens and stool cards, for bacterial taxonomic classification and to allow quantification of the abundance of antimicrobial resistance genes in pre- and post-treatment samples and assess quantitative changes in the relative abundance of antibiotic-resistant bacteria (resistome) over time. Taxonomic and functional profiling of gut microbial communities will be performed using bioinformatics. Comparisons of the resistome by nutraceutical arm, region of travel and incident diarrhea will be performed. We will also evaluate microbiome features in participants with and without TD and resistance gene acquisition to identify predictors of resistance against these endpoints.

Proteomic Profiling on Paired Sera and Enteropathogen Serology
Proteomic profiling of TD cases (using pre- and post-treatment sera) will facilitate identifying biomarkers associated with TD and Functional Bowel Disorder (FBD). Selected biomarkers will be chosen based upon evidence of their association with chronic sequelae of gastrointestinal infection or as a discriminatory marker for symptomatically similar disorders (i.e., celiac disease and inflammatory bowel disease). The biomarkers to be examined may include Anti-Tissue Transglutaminase (tTG), *E. coli* outer-membrane porin C marker (Omp-C), Cbir1 flagellin (CBir), Peri-nuclear Anti-Neutrophil Cytoplasmic Antibodies (pANCA), Anti-*Saccharomyces cerevisiae* antibody (ASCA), C-reactive protein (CRP), thyroid stimulating hormone (TSH), Interleukin 8 (IL-8), Interleukin 10 (IL-10), and Interleukin 12. Time and resources permitting, additional markers, including immune and inflammatory markers (TNF-alpha, IL-1, IL-2, IL-6, IL-17, IL-18, IL-4, IL-5, IL-13, IL-33, CDT, and IFN-gamma), may be evaluated for their potential in distinguishing between the study subgroups. All biomarker concentrations will be determined by regression analysis using a standard curve. Wilcoxon sign rank will be utilized compare each treatment arm of cytokine and microbiome-directed antibody levels between pre and post sera, and a post-hoc Tukey's test for pairwise comparison for each treatment arm.

Additional testing on serum may include the SomaLogic, Inc platform, using Slow Off-rate Modified Aptamers (SOMAmers) that allow for the quantitative detection of over 1100 proteins. SOMAmers are synthetic affinity binding reagents constructed with amino-acid like side chains and acts as 'synthetic antibodies' with a high degree of sensitivity and specificity. SOMAmers offer an improvement over more traditional antibody-based assays in their stability, uniformity, resistance to freeze/thaw cycles and heat denaturation, the ability to rapidly and cost-efficiently generate customized reagents and provide a significant increase in the number of analytes that can be multiplexed (currently over 1100).

Serologic response to pathogens may also be measured using indirect ELISA. Bacterial antigens (*Shigella*, *Campylobacter*, ETEC LT toxin and ETEC-colonization factors [CFA/I, CS3, CS6]), will be used to coat ELISA plates as appropriate. Serum samples, containing antibodies, will be added to coated plates followed by specific conjugate according to antibody isotype to detect (i.e., rabbit anti-human-IgA, IgM and IgG-horseradish peroxidase). Sandwich ELISA will be used for viral pathogen detection (e.g., norovirus); the norovirus antigen being sandwiched between the capture and detected monoclonal antibody.

10.2 Data Collection:

Describe all the data variables, information to be collected, the source of the data, and how the data will be operationally measured.

See Data Collection forms for specifics.

Study data will be collected at the study site(s) and maintained in a standardized format. Data will be collected and entered in an encrypted web-based database using REDCap™ Research Electronic Data Capture software. This database will be accessible through any electronic device with internet access using a secure web browser, with data stored on servers owned and operated by the HJF as part of the IDCRP Data Coordination Center (DCC). Data collection will be completed on an ongoing basis during the study. The methods and instructions for data collection will be provided to the sites after study approval. Any data changes will have an associated audit trail.

The participant diary will be used as a memory aid and will be discussed with study participants at each visit to ensure accuracy and solicit any missing information. The information discussed will then be entered by study personnel directly into REDCap™. REDCap™ may act as a source /CRF; however, paper copies of the case report forms may be used as back-up and will therefore be a source document, when needed.

The Investigator is responsible for assuring that the data collected are complete, accurate, and recorded in a timely manner. Any paper source documentation (the point of initial recording of information) should support the data entered in the electronic case report form in REDCap™ and must be signed and dated by the person recording and/or reviewing the data. Source documents include all recordings of observations or notations of clinical activities, and all reports and records necessary for the evaluation and reconstruction of the clinical trial. Source documents include, but are not limited to, the participant's medical records, laboratory reports, biopsy reports, progress notes, pharmacy records, and any other similar reports or records of procedures performed during participation in the study. Data on case report forms will be collected directly from participants during study visits and telephone calls or will be abstracted from medical records and diary memory aid.

Each study participant will be assigned a unique identification number (ID) at screening that will be used to ensure confidentiality during the study and analysis of their samples. The ID will be included on all questionnaires, study forms, and samples.

The following may act as combination source document/electronic case report form: Administration, Baseline Functional Bowel Disorder Assessment, Demographics, Medical History, Vitals and Physical Exam, Concomitant Medications, Participant Diary, Enrollment, Follow-Up of Functional Bowel Disorder (completed via the web by participants), Initial Symptoms, Specimen Collection, and Study Completion. The following will be case report forms with a separate source document (medical record): Adverse Events, Serious Adverse Events, and Pregnancy Test results. Data from these CRFs will be entered into REDCap™.

A Data Sharing Agreement (DSA) will be executed with the Defense Health Agency (DHA) in order to access electronic medical records of enrolled participants, as well as query the MDR for diagnoses of function bowel disorders.

10.3 At any point in the study, will you request, use, or access health information in any form, including verbal, hard copy and electronic?

(●) Yes ( ) No

10.4 Review the definitions below and respond to the following two questions. If you are not sure of the answers, email DHA.PrivacyBoard@mail.mil for assistance. The *Military Health System (MHS)* is defined as all DoD health plans and DoD health care providers that are organized under the management authority of, or in the case of covered individual providers, assigned to or employed by, the Defense Health Agency (DHA), the Army, the Navy, or the Air Force *MHS workforce members* are employees, volunteers, trainees, and other persons whose conduct, in the performance of work for the MHS, is under the direct control of the MHS, whether or not they are paid by the MHS. *MHS business associates* are persons or entities that provide a service to the MHS and require protected health information (PHI) to provide the service.

Are you an MHS workforce member?

(●) Yes, I am an MHS workforce member
( ) No, I am not an MHS workforce member

Are you an MHS business associate?

( ) Yes, I am an MHS business associate
( ) No, I am not an MHS business associate

10.5 Have you consulted with an MHS data expert to determine the data elements required for your study?

Consulting with a data expert often saves time later in the compliance process because the data expert can advise on the data available in the numerous MHS information systems, the quality of that data and the methods for encrypting and collapsing data. To schedule a consult with an MHS data expert, send an email to: (**DHA.PrivacyBoard@mail.mil**)

( ) Yes, then complete the questions below according to the data consult
(●) No, then complete the questions below according to the best of your knowledge

10.6 Indicate how you will request data from the MHS. Select all that apply.

[x] Talking with MHS health care providers or MHS health plans about specific research participants
[ ] Obtaining MHS hard copy records specific to research participants
[x] Obtaining data from an MHS information system(s)

10.7 If you are obtaining data from an MHS information system(s), indicate whether you plan to receive a data extract or whether you plan to access an MHS information system directly to create a data set.

A data extract is when the MHS or a contractor provides the data set directly to the researcher. When receiving a data set through data extract, the researcher may indicate whether the data elements should be provided as is, encrypted or collapsed. In contrast to a data extract, access to an information system means that the researcher may directly access an MHS information system and create a data set for the research study

[x] Data Extract
[x] Access

10.8 Do you intend to request de-identified data from the MHS in your research study?

There are different two methods for de-identifying data pursuant to HIPAA:

1) Safe Harbor Method: Removing all of the identifiers listed in Table 1 below, provided that the researcher does not have actual knowledge that the remaining data can be used alone or in combination with other information to identify the individual who is the subject of the information
2) Statistical Method: An expert, with appropriate knowledge of and experience with generally accepted statistical and scientific principles and methods for rendering information not individually identifiable, determines that the data is not individually identifiable

○ Yes ◉ No

10.9 Indicate the MHS information system(s) from which you will seek to obtain data

If you do not know which system(s) contains the data elements you need, refer to the Guide for DoD Researchers on Using MHS Data or request guidance from an MHS data expert at: **DHA.PrivacyBoard@mail.mil**.

Below is a list of commonly used MHS systems. If the system from which you seek to obtain data is not listed below, list the name of the system in the "Other MHS Systems" category below
PHI Systems:

MHS Information System	Requesting Data
: AHLTA	: No
: DEERS	: No
: MDR	: Yes
: CHCS	: No
: TMDS	: No
: ESSENTRIS	: No
: MHS Genesis	: No

PII-Only Systems:

MHS Information System	Requesting Data
DMDC	: No

De-Identified Data & Other Systems:

Information System	Requesting Data
No results found	

10.10 Do you intend to merge or otherwise associate the requested data with data from any sources outside of the MHS, including other DoD systems that are not part of the MHS?

◉ Yes, will merge data
○ No, will not merge data

10.11 Indicate the data elements about research participants or relatives, employers, or household members of the research participants that you will request from MHS hard copies or from MHS information systems.
If you will merge data, also indicate non-MHS data elements about research participants or relatives, employers, or household members of the research participants that you will have access to in any form or medium.

Data Element(s)	MHS	Non-MHS Systems	MHS Hard Copies
1. Names	☑	☑	☐
2. Postal address with only town, city, state and zip code	☑	☐	☐
3. Postal address with all geographic subdivisions smaller than a state, including street address, city, county, precinct, zip code and their equivalent geocodes, except for the initial three digits of a zip code if, according to the current publicly available data from the Bureau of Census: 1) the geographic unit formed by combining all zip codes with the same three initial digits contains more than 20,000 people; and 2) the initial three digits of a zip code for all such geographic units containing 20,000 or fewer people is changed to 000	☐	☐	☐
4. Dates including all elements (except year) directly related to an individual, including birth date, admission date, discharge date, and date of death	☑	☑	☐

5. Ages over 89 and all elements of dates (including year) indicative of such age, unless you will only request a single category of "age 90 or older"	☐	☐	☐
6. Telephone numbers	☐	☑	☐
7. Fax numbers	☐	☐	☐
8. Electronic mail addresses	☐	☑	☐
9. Social Security numbers (SSNs)	☐	☐	☐
10. Medical record numbers	☑	☐	☐
11. Health plan beneficiary numbers	☐	☐	☐
12. Account numbers	☐	☐	☐
13. Certificate /license numbers	☐	☐	☐
14. Vehicle identifiers and serial numbers, including license plate numbers	☐	☐	☐
15. Device identifiers and serial numbers	☐	☐	☐
16. Web Universal Resource Locators (URLs)	☐	☐	☐

17. Internet Protocol (IP) address numbers	☐	☐	☐
18. Biometric identifiers, including finger and voice prints	☐	☐	☐
19. Full-face photographic images and any comparable images	☐	☐	☐
20. Any other unique identifying number, characteristic, or code (Diagnosis, DEERS ID, EDI-PI, Rank)	☑	☑	☐

If you are obtaining SSNs, provide a justification as to why and explain why a substitute cannot be used

10.12 Do you believe it is possible for the MHS data to become identifiable because of triangulation, a small cell size, or any unique data element(s)?

Triangulation means using different data elements that are not themselves identifiable but that when combined can be used to identify an individual. For example, triangulation would use rank and race together to determine the identity of an individual with a particular health condition.

Small cell size means that there is only a small number of eligible individuals that satisfy the category description. Guidance for acceptable cell size is available from the Centers for Medicare and Medicaid Services. For example, the rank category of four star generals with a particular diagnosis may be less than 30, so the rank category may need to be expanded to include lower ranks.

A unique data element includes any unique features that are not explicitly enumerated in the categories of data in rows 1 – 20 of the table above (in Section 10.10), but that could be used to identify an individual. Unique data elements include characteristics that are not themselves identifying, such as the rank of general or admiral, or a race or gender, but within the context of other information could be identifiable.

- ○ Yes, I believe there is a reasonable possibility the MHS data will become identifiable
- ◉ No, I believe there is no reasonable possibility the MHS data will become identifiable

10.13 Have you completed and uploaded an appropriate HIPAA document (i.e. HIPAA Authorization will be obtained or Waiver/alteration of HIPAA Authorization is being requested)?

- ◉ Yes
- ○ No
- ○ N/A

10.14 Managing Data (Data Management and/or Sharing Plan) and/or Human Biological Specimens for this Study:

Include in this section the plan for acquiring data (both electronic and hard copy), access during the study, data/specimen storage and length of time stored, shipment/transmission, and the plan for storage and final disposition at the conclusion of the study. Describe any data agreements in place for accessing data within and/or outside of your institution (e.g., Data Sharing Agreement, Data Use Agreement, Business Agreements, etc.)

Methods of specimen collection, handling, and processing are described in the respective SOPs. Details of specimen storage, maintenance, and QA/QC are described in their respective SOPs. Stool samples and bacterial isolates from the study will be housed at the site-specific laboratories, under the supervision of the laboratory investigator.

Serum will be processed and stored frozen at each clinical site with batch shipping to a IDCRP specimen repository pending transfer to laboratory for serology/immune proteomic testing. FTA Micro Elute stool cards will be sent to NMCP for storage and testing.

No study document should be destroyed without prior written agreement between the IDCRP, and the PI.

All samples collected under this protocol may be retained indefinitely.

Coded research data and specimens as well as identifiable protected health information will be collected by the overseas sites UK Armed Forces Training Base (Nanyuki, Kenya), Soto Cano Air Base, Honduras, CLDJ, and Fort Magsaysay (Exercise Balikatan 2025, the Philippines). The sites will follow all confidentiality protections and HIPAA requirements. For the post-infectious functional bowel disorders extended follow- up questionnaire, participants' email addresses or phone numbers (for texting) will be entered into the REDCap system to send participants the follow-up questionnaire web link. The IDCRP owns and controls all data entered in or collected by REDCap. REDCap does not share or distribute customer data and processes all data using industry-best security measures designed to prevent unauthorized access and disclosure. Participant email addresses and phone numbers will be visible to individuals at the DCC who are responsible for setting up the questionnaire.

10.15 Managing Data (Data Management and/or Sharing Plan) and/or Human Biological Specimens for Future Research:

If the study involves collecting, storing, or banking human specimens, data, or documents (either by the Investigator or through an established repository) for FUTURE research, address. How the specimens/data will be used, where and how data/specimens will be stored (including shipping procedures, storage plan, etc.), whether and how consent will be obtained, procedures that will fulfill subjects' request as stated in the consent, whether subjects may withdraw their data/specimens from storage, whether and how subjects may be recontacted for future research and given the option to decline, whether there will be genetic testing on the specimens, who will have access to the data/specimens, and the linkage, the length of time that data/specimens will be stored and conditions under which data/specimens will be destroyed.

Data collected through this protocol will be stripped of participant identifiers for entry into the data management system and be maintained with highly limited access. During the consent process, participants will indicate on the Informed Consent Document whether they grant permission to utilize health information and/or biological specimens for future research. Much of the subsequent research using the materials collected under the auspices of this study will be accomplishable through approved exempt or non-human use sub-studies. All research using the materials collected under this protocol will be conducted in accordance with an IRB-reviewed protocol. Data and samples released for research use will be labeled by participant ID only; data will be shared without any identifiers or links to individuals. Additionally, participants may decide at any point not to have their samples and data stored any longer or used for future research. In this case, the PI will assure the destruction of all known remaining samples and report the destruction to both the participant and to all participating IRBs/REC. This decision may not affect the participant's participation in this protocol. Residual samples will be preserved for future analysis indefinitely, acknowledging that any analysis outside of the scope of this protocol will be requested through each IRB/REC. Exploratory objectives/initiatives are included in what a participant consents to at enrollment and is not considered "future research."

The storage (bank) area for blood specimens is maintained at the IDCRP Specimen Repository. While this study is on-going, stool samples will be handled in accordance with this study's protocol and applicable regulations at the following laboratory: Naval Medical Center Portsmouth, 620 John Paul Jones Circle, Building 3, 1st Floor, Portsmouth, VA 23708.

11.0 Statistical/Data Analysis Plan

11.1 Statistical Considerations:

List the statistical methods to be used to address the primary and secondary objectives, specific aims, and/or research hypotheses. Explain how missing data and outliers will be handled in the analysis. The analysis plan should be consistent with the study objectives. Include any sub-group analyses (e.g., gender or age group). Specify statistical methods and variables for each analysis. Describe how confounding variables will be controlled in the data analysis

We cover the main statistical considerations in this section – further details are delineated in the Statistical Analysis Plan.

11.2 Sample Size:

Up to 300 participants are planned for enrollment in order that 150 participants may be randomized.

11.3 Total number of subjects requested (including records and specimens):

150

11.4 If you are recruiting by study arm, please identify the arms of the study and how many subjects will be enrolled in each arm

11.5 Please provide a justification for your sample size

The non-inferiority of rifaximin to azithromycin will be evaluated using the TLUS. As can be seen in the table below, the median TLUS for standard of care regimen of single dose azithromycin plus loperamide is estimated at 15 hours. This is based on prior observations in the Treat TD trial when restricting to participants with more severe TD illness at presentation. Notably, this is significantly longer than the 4-6 hour median seen when not restricting the population to individuals with functional impairment at time of presentation. Different rifaximin TLUS estimates are included in the table to assess sample size requirements given the below stated assumptions. The threee TLUS for rifaximin are on average 6, 8, and 12 hours longer than the expected recovery period for a participant treated with azithromycin. A recovery period up to 8 hours longer is well within the same 24 hour period and clinically supports the goal of an acceptable treatment regimen for clinical guidance and, from a clinical and operational perspective, is within a time frame that would reasonably be considered truly noninferior.

From the statistical perspective, the 8-hour difference in recovery estimate (the middle column in the table), testing the hazard ratio using the Cox proportional hazards regression model requires 72 participants in group 1 and 72 participants in group 2 to achieve 80.03% power at the 0.05 significance level for an actual hazard ratio of 1 and a non-inferiority hazard ratio of 1.53, assuming the total number of events achieved is 137. The sample size assumes that the incidence rate of the event over the course of the study will be 0.95 and 0.95 in group 1 and group 2 respectively. These results assume that the hazard ratio is constant throughout the study as per the assumptions of the Cox proportional hazards regression model and that higher hazard ratios are assumed to be worse. Given that the evaluable study population for the primary efficacy outcome will be participants followed through study day 7, the loss to follow-up is expected to be minimal. A 10% loss-to-follow-up brings the total study population to 150 (75 participants in each study arm).

Non-inferiority Testing of Two Survival Curves using Cox Regression

Test significance Level, α	0.05	0.05	0.05
Probability of event in Group 1, q1	0.95	0.95	0.95

Probability of event in Group 2, q2	0.95	0.95	0.95
Expected Hazard Ratio, h	1.00	1.00	1.00
Non-inferiority Hazard Ratio, hi	1.40	1.53	1.80
Duration of follow-up, t (in hours)	72	72	72
Group 1 (Azithromycin) Median Survival (= TLUS) (in hours)	15.0	15.0	15.0
Lambda1 (ln(2)/median survival)	0.046	0.046	0.046
Group 2 (Rifaximin) Median Survival (= TLUS) (in hours)	21.0	23.0	27.0
Lambda2 (ln(2)/median survival)	0.033	1.53	1.80
Sample Size Ratio, n2/n1	1.00	1.00	1.00
Group 1 Sample Size, n1	115	72	38
Group 2 Sample Size, n2	115	72	38
Power (%)	80.0	80.3	80.3
Total Number of Events, E	218	137	72

11.6 Data Analysis Plan: Complete description: Background, Objectives, Design, Step by Step how the project is going to be done, Data analysis plan:

Study Population and Analysis Data Sets
Intent-to-Treat/Full Analysis Set Participants: Trial participants receiving any study-specified antibiotic dose with or without use of concomitant medications, and with any amount of follow-up after initial treatment will be included.

Per-Protocol (PP) Evaluable Participants: Trial participants with moderate to severe acute diarrhea impacting activity receiving follow-up to 3 days after antibiotic dose with no use of concomitant medications likely to affect the clinical course.

Safety Evaluable Participants: Trial participants receiving any follow-up after antibiotic dose, regardless of duration, analyzed 'as-treated' for evaluation of any safety or tolerability measures in the trial.

Statistical Analysis - Overview

Statistical Analysis of Clinical Efficacy Outcomes
Participant baseline characteristics and summary follow-up findings will be compared using analysis of variance and other general linear model application methods, Mann-Whitney U-statistic and Kruskal-Wallis tests, and chi-square tests or the exact counterparts (Fisher's, Freeman-Halton, Barnard's), as appropriate. Differences in recovery times will be evaluated using Kaplan-Meier analyses (time to last diarrheal stool), log-rank (overall differences in response curves), and generalized Wilcoxon tests (response curve differences emphasizing early failures). Differences in the frequencies of clinical cures and microbiologic eradication rates between study regimens will be tested for significance using chi-square tests, or the exact counterparts as needed for modest subgroups.

Additional analyses:
Point estimates and 95% confidence intervals for binary outcomes, to include the proportion of participants meeting clinical cure at 24 hours, will be generated using a normal approximation to the binomial distribution or exact (Clopper-Pearson) binomial confidence intervals, as indicated. Logistic regression models may also be developed to evaluate factors associated with clinical outcomes that represent binary endpoints. Rates of adverse reactions will be similarly compared.

Time-to-Event Outcomes:
In addition to using Kaplan-Meier estimates of the survival curve for recovery times (time to last diarrheal stool), time-to-event outcomes will be evaluated using the generalization to log-rank statistics, score statistics from fitted Cox regression models, when adjusting for potential confounders or effect modifiers.

Continuous Outcomes:
Continuous outcome measures that meet regression assumptions will be analyzed using general linear models such as analysis of variance (ANOVA) for analyzing single outcomes across grouping factors, as well as more general linear mixed models for analyzing longitudinal (repeated measures) outcomes; using PROC MIXED.

Statistical Analysis of Post-infectious Functional Bowel Disorders:

The proportion of participants developing post-infectious functional bowel disorders will be estimated along with 95% confidence intervals generated using a normal approximation to the binomial distribution or exact (Clopper-Pearson) methods. Risk factors associated with post-infectious functional bowel disorder risk will assessed using logistic regression models and will include an evaluation of demographic characteristics (e.g., sex, age, ethnicity, etc.) as well as acute illness characteristics (e.g., pre-treatment illness duration, total illness duration, disease severity, pathogen identified, TD syndrome presentation, treatment, response to treatment, etc.).

12.0 Participant Information

12.1 Subject Population:

Active duty military personnel deployed to Kenya (UK military) or Honduras or Djibouti or the Philippines (US military) ages 18-60 years presenting with acute watery diarrhea who are deemed suitable for ambulatory therapy. Children and others who cannot provide informed consent will not be included in the trial.

12.2 Age Range:

Check all the boxes that apply. if the age range of potential subjects (specimens, records) does not match the range(s) selected, please specify in the text box.

- [ ] 0-17
- [x] 18-24
- [x] 25-34
- [x] 35-44
- [x] 45-54
- [x] 55-64
- [ ] 65-74
- [ ] 75+

ages 18-60

12.3 Gender:

- [x] Male
- [x] Female
- [x] Other

12.4 Special categories, check all that apply

- [ ] Minors /Children
- [ ] Students
- [ ] Employees - Civilian
- [ ] Employees - Contractor
- [ ] Resident/trainee
- [ ] Cadets /Midshipmen
- [x] Active Duty Military Personnel
- [ ] Wounded Warriors
- [ ] Economically Disadvantaged Persons
- [ ] Educationally Disadvantaged Persons
- [ ] Physically Challenged (Physical challenges include visual and/or auditory impairment)
- [ ] Persons with Impaired Decisional Capacity
- [ ] Prisoners

☐ Pregnant Women, Fetuses, and Neonates
☐ Non-English Speakers
☐ International Research involving Foreign Nationals - Headquarters Review is necessary

You must also consider the requirements of DoDI 3216.02, Enclosure 3, paragraph 7.e.

12.5 Inclusion Criteria:

Order Number	Criteria
1	Active duty military, 18-60 years old
2	Presence of acute watery diarrhea (3 or more loose/liquid stools in 24 hours or 2 loose/liquid stools in 24 hours and associated symptoms such as nausea, vomiting, abdominal cramps or tenesmus) lasting <96 hours in duration **that impacts function in activities and/or performance of primary duties.** Refer to recruitment decision tree below.
3	Eligible for ambulatory management. (Note: Ambulatory management in this study means that the patient can be treated on an outpatient basis to include up to 23 hours of medical observation and IV therapies.)
4	Able to comply with follow-up procedures.
5	Will remain in country for at least 7 days or an assurance that follow-up can be arranged elsewhere.

12.6 Exclusion Criteria:

Order Number	Criteria
1	Allergy to rifamycins, macrolides, or loperamide (not including mild gastrointestinal upset).
2	Antibiotic therapy in the 72 hours prior to presentation (excluding malaria prophylaxis with mefloquine, malarone, chloroquine/proguanil, tafenoquine, or doxycycline).
3	Concomitant medications with known drug-drug interactions with any of the study drugs (includes theophylline, digoxin, warfarin, chloroquine and hydroxychloroquine).
4	Current or history of liver disease or other serious health conditions based on review by study physician.
5	Acute dysentery and/or febrile illness (temperature > 100.4ºF[38.1ºC]).
6	Presence of symptoms >96 hours prior to initiating treatment.
7	Use of >4 mg loperamide or use of any amount of loperamide for greater than 24 hours prior to enrollment. Prior use of bismuth subsalicylate or other anti-diarrheal, non-antibiotic therapy in the 48 hours prior to enrollment.
8	Positive pregnancy test at presentation (unknown effects with rifaximin). All female participants will be administered a urine pregnancy test prior to enrollment.

9	Previously screened or randomized in this study.

13.0 Recruitment and Consent

13.1 Please describe the recruitment process, including how subjects will be identified and selected for the study.

Identification and Selection of Subjects

Participant recruitment will be equitable but will be limited to active duty present at the study sites. The study aims to answer questions regarding the management of TD in this specific population. Eligibility will be based solely on the inclusion/exclusion criteria outlined above. Only individuals meeting criteria in the Deployment Health Guidance as requiring antibiotics (i.e., those with moderate to severe acute watery diarrhea that impacts performance) will be enrolled. We will not include children or individuals with developmental or other disabilities.

Recruitment Process

For all sites, study enrollment is NOT a condition of treatment and patients who do not enroll will be treated by a clinician according to site-/country-specific standards of care.

The trial will take place at 4 individual site locations: British Army Training Unit, Kenya (BATUK), Soto Cano Air Base, Honduras, Camp Lemonnier, Djibouti (CLDJ), and the Fort Magsaysay Role 2E medical facility during the Balikatan 2025 exercise. These sites were chosen because they were high enrolling sites with TrEAT TD, they have had high rates of travelers' diarrhea, and/or they are fixed facilities with the necessary infrastructure in place to support this trial. US research teams will recruit, enroll, and follow US participants and UK research teams will recruit, enroll, and follow UK participants. Recruitment will include referral by treating physician.

To maximize enrollment and results, a variety of strategies may be implemented including providing a handout and hanging posters around base to advertise the study, in addition to electronic dissemination of study flyers.

BATUK is a permanent administrative and training installation in Nanyuki, Kenya. The base supports 8 different 6-week battalion rotations consisting of 1000-1200 personnel along with a permanent party of 100-150 staff. BATUK currently does not have in-patient beds. BATUK is staffed by 2 family practice physicians (one military officer and one civilian) each unit provides their own medical support, which includes at least one experienced military medical officer. Patients presenting with diarrhea will be seen by an experienced combat medical technician (CMT), and if symptoms warrant during off-hours, an on-call physician will be consulted. At this point, the patient will be approached for enrollment by a research coordinator or study clinician. If the patient agrees to enroll, he/she will be taken through the enrollment procedure by a member of the study team (CRC or physician). Follow-up will occur locally at 1 of the 2 clinic sites and recorded per protocol. Recruiting UK military deploying to Kenya, with an established laboratory, capable of frozen storage of stool and serum samples, would provide useful medical intelligence on pathogen etiology and antibiotic resistance.

Soto Cano AB is an expeditionary base located in central Honduras. The base has a population of approximately 600 active duty military personnel assigned on 1 year, 6-month or 90-day orders. Personnel deploy at various times throughout the year on a rolling basis with major transitions in the base population during the summer and winter months. An on-site medical element staffed by active duty and reserve personnel and several local physicians provide year-round medical care to military beneficiaries. Personnel stationed at Soto Cano AB have access to the local economy via liberty and their official duties. Anecdotal rates of TD are high. For study purposes, the medical clinic will be augmented by a rotation of credentialed providers and other study personnel.

Camp Lemonnier is a US Navy Expeditionary Base located in Djibouti, on the Horn of Africa. The base has a population of 3,200 both active duty and contractors. Camp Lemonnier serves as AFRICOM's only fixed military base. Naval Medical Research Unit-EURAFCENT (NAMRU-EURAFCENT) has provided support for Camp Lemonnier by providing diarrheal surveillance. The Expeditionary Medical Facility (EMF) clinic is fully staffed by US Navy military personnel and is always open for sick call and emergencies for US forces. For study purposes, a container working unit (CWU) was built initially in support of the Treat TD trial and is still in use by NAMRU-EURAFCENT staff. The CWU resides next to the EMF. The CWU will be staffed by a rotation of credentialed providers.

Exercise Balikatan is the largest annual bilateral training exercise conducted between the U.S. and the Philippines. The three-week exercise is held at locations throughout the Philippines and consists of approximately 10,000 U.S. service members. A team of active-duty study credentialed providers will be deployed to staff and recruit at the Fort Magsaysay Role 2E medical facility which has a catchment area of approximately 2,000 U.S. service member during Exercise Balikatan.

Patients at all sites will be assessed by a study clinician to assure that they are hemodynamically stable, in no distress, and meet the definition of diarrhea. Through clinical interview with the patient, the clinician will utilize the flow diagram below to determine whether the patient's illness impacts their activity/ability to function, thereby meeting the guidelines regarding use of antibiotics for treatment.

Figure. Recruitment Decision Tree

If the patient's illness meets one of the "Recruit for trial" categories, the study clinician will introduce the study to the patient. If the patient is interested in the study, the study clinician or clinical trial

coordinator will complete the informed consent process with the patient. After that occurs, the full study eligibility will be determined. Patients not wishing to participate in the study will be offered standard site-/country-specific care by the study or non-study health care provider. If the patient has multiple complaints, these providers will respond to their other complaints with standard evaluation and treatment consistent with the operating procedures of their practice. The patient's medical encounter for TD and enrollment into the trial will be documented using the standard medical record documentation for the clinical site (e.g., paper-based note in their medical record, or electronically captured encounter).

Due to the operational considerations involving urgency associated with diarrhea and because it may be necessary to collect for clinical purposes, the study will allow for stool collection within 2 hours prior to informed consent. The stool sample will not be taken possession of as part of the study until the participant signs the consent document. Furthermore, no processing or testing of the stool specimen for research will be conducted until the participant is appropriately consented. The research staff will perform a visual inspection of the stool as well as perform a hemoccult test on the sample if suspected blood is visualized in stool. A person presenting with febrile (meeting the stated fever definition) diarrhea with or without dysentery will be exclusionary.

13.2 Compensation for Participation:

Participants will be eligible to receive a maximum compensation of $250.00, in the form of Amazon gift codes, for participation in this study. The compensation will be administered in three $50.00 increments in accordance with 24 USC 30, which will be given to each volunteer who participates in follow-ups associated with blood sample collection on Days 0, 7, and 21; compensation for self-collected samples associated with virtual visits will be the same as blood collected in clinic. Participants will receive $25.00 for the completion of the Days 1 and 3 clinical follow up visits each. Additionally, participants will receive $50.00 for the 3 month follow-up questionnaire (in accordance with DoDI 3216.02, US participants must be off-duty at the time the Day 1 and Day 3 clinical visits as well as the time that the 3 month questionnaire is completed in order to be compensated).

13.3 Please describe the pre-screening process. If no pre-screening, enter Not Applicable in the text editor

N/A

13.4 Consent Process: Revised Common Rule, Section 219.116: General requirements for informed consent, whether written or oral, are set forth in this paragraph and apply to consent obtained in accordance with the requirements set forth in paragraphs (b) through (d) of this section. Broad consent may be obtained in lieu of informed consent obtained in accordance with paragraphs (b) and (c) of this section only with respect to the storage, maintenance, and secondary research uses of identifiable private information and identifiable biospecimens.

Are you requesting a waiver or alteration of informed consent?

○ Yes ◉ No

Please explain the consent process:

Prior to study entry, patients who present for care with a chief complaint of acute watery diarrhea will be told by a member of the clinic staff (potentially front desk) unaffiliated with the study that they may be eligible for a research study being conducted at the site. Patients who are not interested will either be seen by clinicians unaffiliated with the study or study clinicians, depending on availability. If they agree to learn more, the potentially eligible individual will be approached by study personnel and given a detailed explanation of the study objectives and requirements for participation using the Participant Information Sheet (for UK servicemembers) or Informed Consent Document (for US servicemembers). After the explanation is completed, potential participants will be given the opportunity to ask questions about the study and clarify points or receive any additional information they desire. The study clinician or research coordinator will provide the participants with a signature page (for UK servicemembers) to read and sign or will ask them to sign consent form if they are a US servicemember. Potential

participants will be informed that their participation in the study is voluntary and persons who elect not to participate will be treated according to site-/country-specific standards of care. The consent form and HIPAA (US) authorization will be photocopied or signed in duplicate with one copy being given to the participant and the original being maintained at the study site in a secure file. The consent process will be conducted in private, consistent with the privacy afforded for medical visits at the facility. Coercion will be minimized with provision of a clear informational brief which states that their willingness to participate is completely voluntary and that they will receive the standard of care regardless of participation in the study. Per Department of Defense Instruction 3216.02, superiors of service members in the chain of command will not be present during recruitment or the consent process. Service members will be recruited and consented individually; therefore, an ombudsman will not be employed for the study.

Participants will be allowed to collect a stool sample as part of the Day 0 procedures within 2 hours prior to consent if necessary due to urgency.

13.5 DoDI 3216.02 requires an ombudsman to be present during recruitment briefings when research involves greater than minimal risk and recruitment of Service members occurs in a group setting. If applicable, you may nominate an individual to serve as the ombudsman.

- (●) N/A
- ( ) Propose ombudsman

13.6 Withdrawal from Study Participation:

Explain the process for withdrawal and specify whether or not the subjects will be given the opportunity to withdraw their data their data/specimens in the event they wish to withdraw from the study

A participant may voluntarily end participation in the study at any time. An individual's participation in the study may be stopped if the participant chooses to withdraw, can no longer meet follow-up requirements, or an investigator chooses termination to be in the best interest of the participant. If a participant withdraws, study staff will make a reasonable effort to determine the reason for the participant's withdrawal from the study. Telephone calls and/or e-mail correspondence will be considered reasonable effort. All data and specimens collected up to the time of withdrawal will be reported and retained.

Participants may decide at any point not to have their samples and data stored any longer or used for future research. In this case, the PI will assure the destruction of all known remaining samples and report what was done to both the participant and to all participating IRBs. This decision may not affect the participant's participation in this protocol.

14.0 Risks and Benefits

14.1 Risks of Harm:

Identify all research-related risks of harm to which the subject will be exposed for each research procedure or intervention as a result of participation in this study. Consider the risks of breach of confidentiality, psychological, legal, social, and economic risks as well as physical risks. Do not describe risks from standard care procedures; only describe risks from procedures done for research purposes

Study Drugs – expected adverse events are listed/described in the package inserts of the respective dugs. For the study antibiotics they include allergic reactions, rash, nausea, vomiting, stomachache,

headache, flatulence, diarrhea/loose stools, rectal tenesmus, defecation urgency, dyspepsia and vaginitis. The primary risks associated with the use of loperamide (an over-the-counter anti-motility agent) are constipation and abdominal cramp.

Study Procedures
Secondary infection and syncope from vasovagal reactions occur from blood collection. Bruising and bleeding may occur because of blood collection procedures.

There are no expected adverse events related to the stool collection.

A physician is available at each site to monitor these events. Any adverse event resulting from blood collection procedures will be managed according to standard clinical procedures.

14.2 Measures to Minimize Risks of Harm (Precautions, safeguards):

For each research procedure or intervention, describe all measures to minimize and/or eliminate risk of harms to subjects and study personnel

As previously mentioned in the study procedures, the participants will be asked to follow-up at 24 hours (+/- 6 hours), 72 hours (+/- 12 hours), and 7 days (+/- 2 days) after entry into the study to perform a clinical assessment (and may include an interview regarding symptoms associated with illness, a physical exam, collection of vital signs, and repeated pregnancy testing for females of childbearing potential where the principal investigator or designee is uncertain of the pregnancy status). Participants will be given diaries to complete daily. Information to be included on the diary includes time of loperamide usage, the number and form of all stools passed as well as the presence of enteric symptoms, disability, and global wellness assessments. The diary cards, which will act as memory aids, will be reviewed by the investigator with the participant during each follow-up clinic visit until wellness is achieved, for a minimum of 3 days. Additional follow-up at 21 days (- 7/+14 days) will be conducted to monitor changes in bowel flora. Use of concomitant medications and occurrence of adverse events will be assessed at all follow-up visits.

14.3 Confidentiality Protections (for research records, data and/or specimens):

Describe in detail the plan to maintain confidentiality of the research data, specimens, and records throughout the study and at its conclusion (e.g., destruction, long term storage, or banking). Explain the plan for securing the data (e.g., use of passwords, encryption, secure servers, firewalls, and other appropriate methods). If data will be shared electronically with other team members/collaborators outside the institution, describe the method of transmission and safeguards to maintain confidentiality. Explain whether this study may collect information that State or Federal law requires to be reported to other officials or ethically requires action, e. g., child or spouse abuse

Participants will be identified on data collection forms and serum and stool specimens by a unique participant identification number. The code linking study ID to personal identifiers will be kept at the study site on a password protected file on a computer that requires a password to access. Access to the link will be restricted to study personnel requiring the information for study procedures. Paper records and consent documents will be kept in a locked area with restricted access at each research site. Email address and phone number (for use in sending out the 3-month questionnaire) will be accessible by data management staff requiring it as part of their role. No personal identifiers will be transferred to the study database or to data analysis study personnel. No personal identifiers will be used in any publication or communication used to support this research study. Individuals responsible for quality

assurance, MODREC, USU IRB, and The Henry M. Jackson Foundation for the Advancement of Military Medicine, Inc. are eligible to review medical and research records related to this study as a part of their responsibility to protect human participants in clinical research. Medical and research records will be de-identified before photocopying.

Each site PI will be responsible for retaining sufficient information about each participant, i.e., name, address, telephone number, and identity in the study, so that the regulatory authorities may have access to this information should the need arise.

Study files retained at the individual study sites will be stored in the research spaces in secured cabinets, secured cases, or secured rooms with restricted access.

Document Retention: Standards herein will be applied to both paper files and digital files (including certified digital copies of paper source documents). If certified copies are produced of paper source documentation, the original paper documentation does not need to be retained. Storage of documentation at offsite facilities is permitted.

Applicable Site or Core IRB closure will be completed prior to destruction of source documents including HIPAA Authorizations and Regulatory Essential Documents. Source Documents will be retained for at least:

- 3 years after completion of data collection activities and
- 6 years after last use of the HIPAA authorization for HIPAA Authorizations and Regulatory Essential Documents

Data will be stored for as long as necessary to ensure the ability to respond to questions regarding published results.

14.4 Potential Benefits:

Describe any real and potential benefits of the research to the subject and any potential benefits to a specific community or society

If the individuals in the research are considered experimental subjects (per 10 USC 980), and they cannot provide their own consent, the protocol must describe the intent to directly benefit all subjects

The proposed study aims to compare two licensed antibiotics, both of which have extensive use for the treatment of TD. The trial may provide the evidence to support expanding the current first-line options for deployed military healthcare. In addition, to the military healthcare benefit, individuals may benefit from a safe and effective treatment for ambulatory watery diarrhea that has the potential to lessen post-infection sequelae and limit the promotion of resistant Gram-negative bacterial pathogens. The benefits outweigh the risks in this study particularly due to the active safety and clinical monitoring post-enrollment.

14.5 Privacy for Subjects:

Describe the measures to protect subject's privacy during recruitment, the consent process, and all research activities, etc.

The consent process will be conducted in private, consistent with the privacy afforded for medical visits at the facility. Coercion will be minimized with provision of a clear informational brief which states that their willingness to volunteer is completely voluntary and they will receive the standard of care

regardless of participation in the study. Per Department of Defense Instruction 3216.02, superiors of service members in the chain of command will not be present during recruitment or the consent process. Service members will be recruited and consented individually; therefore, an ombudsman will not be employed for the study.

14.6 Incidental or Unexpected Findings:

Describe the plan to address incidental findings and unexpected findings about individuals from screening to the end of the subject's participation in the research. In cases where the subject could possibly benefit medically or otherwise from the information, state whether or not the results of screening, research participation, research tests, etc., will be shared with subjects or their primary care provider. State whether the researcher is obligated or mandated to report results to appropriate military or civilian authorities and explain the potential impact on the subject

No incidental or unexpected findings are expected as only laboratory testing related to the diarrheal illness and/or impact of the illness on the gut will be conducted.

15.0 Study Monitoring

15.1 Your study requires either Data and Safety Monitoring Plan (DSMP) or a Data and Safety Monitoring Board (DSMB).

- ○ DSMP
- ○ DSMB
- ◉ Both
- ○ Not Applicable

A DSMP should describe the plan to monitor the data to verify that the data are collected and analyzed as specified in the protocol. Include who will conduct the monitoring, what will be monitored, and the frequency of monitoring. It should also include the plan to ensure the safety of subjects

A Quality Management Plan will be utilized that covers Quality Control procedures that will occur at the site as well as Quality Assurance measures that will be conducted by members of the study's project coordination group. For Quality Assurance, personnel may conduct virtual or in-person visits to monitor all aspects of the study in accordance with the appropriate regulations and the approved protocol. The objectives of quality assurance will be: 1) to verify the existence of signed informed consent documents and documentation of the informed consent process; 2) to verify the prompt and accurate recording of all monitored data points, and prompt reporting of all SAEs; 3) to compare abstracted information with individual participants' records and source documents (participants' charts, laboratory analyses and test results, physicians' progress notes, nurses' notes, and any other relevant original participant information); and 4) to help ensure study site personnel are in compliance with the protocol.

Describe the composition of your DSMB and how frequently it meets. Explain who will be responsible for ensuring data accuracy and integrity, how often interim data be reviewed - and by whom - and who will perform aggregate analysis of data and adverse events.

A Data Safety Monitoring Board (DSMB) will be chartered that will comprise of the US and UK research monitors (military physicians with relevant expertise in operational medicine) and an independent biostatistician. The role of the DSMB will be to review and concur with the Clinical Protocol and the Statistical Analysis Plan and to review the final study analysis report. No interim analysis will take place unless the study stopping rules are met.

Accruing safety data will be closely monitored by 2 safety review groups: one internal to the study team and the DSMB. The internal group includes the study PI and US and UK Site PIs. The DSMB composition is described above. The DSMB central research monitors will assume the role of site medical safety monitor and will be a physician (or other licensed clinician) with relevant expertise whose primary responsibility is to provide independent safety monitoring in a timely fashion. Participation will last for the duration of the study. The research monitors will have no direct involvement in the conduct of the study, and no financial, proprietary, professional, or other interests that may affect impartial, independent decision-making.

The primary focus of the central research monitors is to independently review all adverse events considered serious and unexpected. Clinical and laboratory data, clinical records, and other study-related records will be made available for review. The PI will ensure that the research monitors are apprised of all new safety information relevant to the study product and the study.

The first DSMB meeting occured prior to study initiation. At the initial meeting, the DSMB was briefed on the protocol and the Statistical Analysis Plan (SAP). The DSMB reviewed and approved the DSMB charter. The DSMB concurrence of the Clinical Protocol, the Statistical Analysis Plan, and the signed DSMB Charter was provided after the initial DSMB meeting which occurred in September 2022.

The DSMB will meet on an ad hoc basis as described in protocol in the event of related SAE, stopping criteria, or unblinding for rescue therapy. Immediate actions for participant safety such as unblinding and rescue therapy do not require waiting for DSMB review; however, the board will be provided participant safety details within 48 hours.

The DSMB will also meet at study completion to review the final clinical study report.

A decision to terminate the study will be made with caution and in the context of all pertinent data. If any of these stopping criteria are met, enrollment among all study sites will be stopped and the DSMB, USU IRB, Ministry of Defence Research Ethics Committee (MODREC), and all participating sites and affiliated commands will be notified. The DSMB will review the information and the decision to terminate the study and provide feedback to the investigative team. All relevant oversight bodies will be consulted prior to resuming the study.

The DSMB will review unblinded safety data on an individual participant basis in the event that the blind is broken for "rescue therapy." No overall unblinded safety data will be reviewed by the DSMB during the course of the study.

16.0 Reportable Events

16.1 Reportable Events: Consult with the research office at your institution to ensure requirements are met. Describe plans for reporting unexpected adverse events and unanticipated problems. Address how unexpected adverse events will be identified, who will report, how often adverse events and unanticipated problems will be reviewed to determine if any changes to the protocol or consent form are needed and the scale that will be used to grade the severity of the adverse event.

Consult with the research office at your institution to ensure requirements are met

• Describe plans for reporting expected adverse events. Identify what the expected adverse events will be for this study, describe the likelihood (frequency, severity, reversibility, short-term management and any long-term implications of each expected event)

• Describe plans for reporting unexpected adverse events and unanticipated problems. Address how unexpected adverse events will be identified, who will report, how often adverse events and unanticipated problems will be reviewed to determine if any changes to the research protocol or consent form are needed and the scale that will be used to grade the severity of the adverse event

Definitions:

Adverse Event (AE): An AE is any untoward or unfavorable medical occurrence in a human participant, including any abnormal sign (e.g., abnormal physical exam or laboratory finding), symptom, or disease, temporally associated with the participant's participation in the research, whether or not considered related to the research. An AE is NOT the symptoms and signs related to

the target disease of interest, acute watery diarrhea, for which the trial is assessing treatment except for incidents of nausea or vomiting that occur during the 30-minute observation period following antibiotic administration.

Adverse Reaction (AR)
An adverse event that is caused by an investigational agent (drug or biologic).

Suspected Adverse Reaction (SAR)
An adverse event for which there is a reasonable possibility that the investigational agent caused the adverse event. 'Reasonable possibility' means that there is evidence to suggest a causal relationship between the drug and the adverse event. A suspected adverse reaction implies a lesser degree of certainty about causality than adverse reaction which implies a high degree of certainty.

Serious Adverse Event (SAE)
A Serious Adverse Event is an AE that results in one or more of the following outcomes:

- death
- a life threatening (i.e., an immediate threat to life) event
- an inpatient hospitalization or prolongation of an existing hospitalization
- a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- a congenital anomaly/birth defect
- a medically important event*

* Medical and scientific judgment should be exercised in deciding whether expedited reporting is appropriate in other situations, such as important medical events that may not be immediately life threatening or result in death or hospitalization but they may jeopardize the participant or may require intervention to prevent one of the other outcomes listed above.

Unexpected Adverse Event
An AE is unexpected if it is not listed in the Investigator's Brochure or Package Insert (for marketed products) or is not listed at the specificity or severity that has been observed. It is the responsibility of the investigator to make this determination.

Serious Unexpected Suspected Adverse Reaction (SUSAR)
A SUSAR is a Suspected Adverse Reaction that is both Serious and Unexpected. The PI must report all SUSAR, regardless of expectedness, that are related to the research to the IRB within 48 hours of awareness.

Unanticipated Problem (UP)
An Unanticipated Problem is any event, incident, experience, or outcome that is

1. unexpected in terms of nature, severity, or frequency in relation to
    1. the research risks that are described in the IRB-approved research protocol and informed consent document or other study documents; and
    2. the characteristics of the participant population being studied; and
2. possibly, probably, or definitely related to participation in the research; and
3. places participants or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

Unanticipated problem that is not an Adverse Event (UP non-AE)
A UP that does not fit the definition of an AE, but which may, in the opinion of the investigator, involve risk to the participant, affect others in the research study, or significantly impact the integrity of research data. Examples include occurrences of breaches of confidentiality, accidental destruction of study records, and unaccounted-for study drug.

Adverse event grading (Severity): All AEs will be assigned severity by the Investigator at the time of evaluation according to the scale as follows:

1. – Normal; No adverse event
2. – Mild; Easily tolerated and does not interfere with normal daily activities
3. – Moderate; Causes some interference with normal daily activities
4. – Severe; Prevents normal daily activities

Relationship (Causality): All AEs will be assigned a relationship to the study investigational product by the Investigator at the time of AE assessment according to the following criteria:

Unrelated meaning either:
Not Related

- Does not follow a reasonable temporal sequence from administration of the treatment medications
- Does not follow a known response pattern to the antibiotic or loperamide
- Can be explained by the known characteristics of the participant's clinical state or other modes of therapy administered to the participant

Unlikely Related

- May or may not follow a reasonable temporal sequence from administration of the treatment medications
- Does not follow a known response pattern to the antibiotic or loperamide
- Can be explained by the known characteristics of the participant's clinical state or other modes of therapy administered to the participant

Related meaning either:
Possibly Related

- Follows a reasonable temporal sequence from administration of the treatment product
- May follow a known response pattern to the treatment product
- May also be reasonably explained by the participant's clinical state or other modes of therapy administered to the participant

Probably Related

- Follows a reasonable temporal sequence from administration of the treatment product
- May follow a known response pattern to the treatment product
- Could not be reasonably explained by the known characteristics of the participant's clinical state or other modes of therapy administered to the participant, if applicable

Definitely Related

- Follows a reasonable temporal sequence from administration of the treatment product
- Follows a known response pattern to the treatment product
- Could not be reasonably explained by the known characteristics of the participant's clinical state or other modes of therapy administered to the participant, if applicable

The following common symptoms associated directly with diarrheal illness and not with participating in the study, will not be considered or reported as adverse events: abdominal cramping, nausea, fever, vomiting, excessive gas, urgency to empty bowel, and malaise/fatigue. Participants will be able to indicate severity for these symptoms experienced in their diary according to the scale above. This participant-assessed severity will be discussed and confirmed by the Investigator at the next diary review (clinic visit).

Documentation and Reporting
Investigators will solicit and record AEs that are at least possibly related to the study medication with moderate or greater severity.

Expected adverse events [from package inserts (azithromycin, rifaximin, and loperamide), as well as those related to blood draws) which are not serious are reported on the Continuing Review Report (included in Risks section). Continuing Review is typically on a 12-month cycle, the anniversary month of the protocol's initial approval or due in lesser than 12-month cycle as determined by the IRB for continuing review and approval.

Serious related adverse events: The PI must report all SAEs, regardless of expectedness, that are related to the research to the IRB within 48 hours of awareness. SAEs that are not related to participation in the research and hospitalization for worsening/relapse of diarrhea/dysentery related to being on rescue therapy will only be reported to the IRB office during continuing review.

Non serious unexpected adverse events that are related to the research must be reported to the IRB within 10 working days of investigator awareness. Non serious unexpected adverse events that are unlikely related or unrelated to the research will be reported during continuing review.

The PI must forward a copy of all reportable adverse events to the research monitor promptly. All serious adverse events and all unexpected adverse events will be reported in the occurrences and timelines described above to MODREC at the same time as the USU IRB submission.

A summary of all serious and non-serious adverse events will be included in the Continuing Review Report.

Reporting Protocol Deviations
A Protocol Deviation is any change, divergence, or departure from the study design or procedures of an IRB-approved research protocol that does not have a major impact on the participant's rights, safety or well- being, and/or the completeness, accuracy and reliability of the study data.

The PI or designee will be responsible for identifying and reporting all deviations, which are defined as isolated occurrences involving a procedure that did not follow the study protocol or study-specific procedure. Investigators will report protocol deviations to the USU IRB within 10 business days that meet any of the following:

1. The deviation has harmed or posed a significant or substantive risk of harm to the research participant,
2. The deviation compromises the scientific integrity of the data collected for the study,
3. The deviation is a willful or knowing breach of human participant protection regulations, policies, or procedures on the part of the investigator(s),
4. The deviation involves a serious or continuing noncompliance with federal, state, local or institutional human participant protection regulations, policies, or procedures,
5. The deviation is inconsistent with DoD, medical, and ethical principles,
6. Any other protocol deviations which, in the opinion of the PI, should be promptly reported to the IRB.

All protocol deviations will also be sent for review and approval to the MODREC. A log of all deviations, including minor deviations that do not meet any of the criteria above, will be reported annually in the continuing review report to the IRB and in the Final Study Report.

17.0 Equipment/non-FDA Regulated Devices

17.1 Does the study involve the use of any unique non-medical devices/equipment?

○ Yes ◉ No

18.0 FDA-Regulated Products

18.1 Will any drugs, dietary supplements, biologics, or devices be utilized in this study?

- [x] Drugs
- [ ] Dietary Supplements
- [ ] Biologics
- [ ] Devices
- [ ] N/A

18.2 Drugs, Dietary Supplements and Biologics/Vaccines details:

- [x] Are drug(s) in this research being used in accordance to the approved labeling?
- [ ] Are drug(s) in this research being used in a manner other than its approved labeling?

Enter Dietary Supplements and Biologics/Vaccines in the Drug Information table. Complete all relevant fields in the table ("Protocol Drug Details" screen). If the question is not relevant, leave the question blank and/or do not change the default selection.

View Details	Drug Name	FDA Approved	A new drug or a new use of an already approved drug:	IND Number
⊟	**Trade Drug Name:** RIFAXIMIN **Generic Drug Name:** **Investigational Drug Name:**	Yes	No	

Trade Drug Name:	RIFAXIMIN
Generic Drug Name:	
Investigational Drug Name:	
Identify the name of the manufacturer or source of investigational drug/biologic:	Salix Pharmaceuticals
Is the drug supplied at no cost?	No
Is the Drug FDA Approved:	Yes
Is this a new drug or a new use of an already approved drug	No
Is an IND necessary	No
IND Number	
Who holds the IND:	N/A
IND details:	
If FDA Approved and an IND is not required, Please provide a rationale for exemption:	
Are you currently using this IND in another research project?	No
If yes, list the IRB Number(s):	

Dose Range:	550 mg
Frequency:	Once
Route of administration:	By mouth
Will the investigational pharmacy be dispensing?	No
If the source is not a FDA licensed facility, provide details regarding the purity, quality, stability and sterility of the investigational drug/biologic:	Purchased through contracted pharmacy.
Identify who will be preparing the investigational drug /biologic for administration and describe in detail how it will be prepared:	Contracted pharmacy.
Indication(s) under Investigation:	
Where will the drug be stored	At each site in a secured, locked cabinet in a locked room.
Drug Storage Restrictions (including temperature, etc.):	Room temperature
Administration Instructions:	See other application sections
Possible Untoward Effects, Their Symptoms & Treatment:	See other application sections
Potential or Actual Antidotes for Excessive or Adverse Drug Effect:	N/A
Contraindications and Interactions, If Known:	See other application sections
Investigators Authorized to Prescribe:	

⊟	**Trade Drug Name:** AZITHROMYCIN **Generic Drug Name:** **Investigational Drug Name:**	Yes	No

Trade Drug Name:	AZITHROMYCIN
Generic Drug Name:	
Investigational Drug Name:	
Identify the name of the manufacturer or source of investigational drug/biologic:	Pfizer
Is the drug supplied at no cost?	No
Is the Drug FDA Approved:	Yes
Is this a new drug or a new use of an already approved drug	No
Is an IND necessary	No
IND Number	
Who holds the IND:	N/A
IND details:	
If FDA Approved and an IND is	

not required, Please provide a rationale for exemption:	
Are you currently using this IND in another research project?	No
If yes, list the IRB Number(s):	
Dose Range:	500 mg
Frequency:	Once
Route of administration:	By mouth
Will the investigational pharmacy be dispensing?	No
If the source is not a FDA licensed facility, provide details regarding the purity, quality, stability and sterility of the investigational drug/biologic:	
Identify who will be preparing the investigational drug /biologic for administration and describe in detail how it will be prepared:	Contracted pharmacy
Indication(s) under Investigation:	
Where will the drug be stored	At each site in a secured, locked cabinet in a locked room.
Drug Storage Restrictions (including temperature, etc.):	Room temperature
Administration Instructions:	See other application sections
Possible Untoward Effects, Their Symptoms & Treatment:	See other application sections
Potential or Actual Antidotes for Excessive or Adverse Drug Effect:	N/A
Contraindications and Interactions, If Known:	See other application sections
Investigators Authorized to Prescribe:	

⊟	**Trade Drug Name:** LOPERAMIDE **Generic Drug Name:** **Investigational Drug Name:**	Yes	No

Trade Drug Name:	LOPERAMIDE
Generic Drug Name:	
Investigational Drug Name:	
Identify the name of the manufacturer or source of investigational drug/biologic:	Johnson and Johnson
Is the drug supplied at no cost?	No
Is the Drug FDA Approved:	Yes
Is this a new drug or a new use of an already approved drug	No

Is an IND necessary	No
IND Number	
Who holds the IND:	N/A
IND details:	
If FDA Approved and an IND is not required, Please provide a rationale for exemption:	See other application sections
Are you currently using this IND in another research project?	No
If yes, list the IRB Number(s):	
Dose Range:	2-4 mg
Frequency:	See other application sections
Route of administration:	By mouth
Will the investigational pharmacy be dispensing?	No
If the source is not a FDA licensed facility, provide details regarding the purity, quality, stability and sterility of the investigational drug/biologic:	
Identify who will be preparing the investigational drug /biologic for administration and describe in detail how it will be prepared:	Contracted pharmacy
Indication(s) under Investigation:	
Where will the drug be stored	At each site in a secured, locked cabinet in a locked room.
Drug Storage Restrictions (including temperature, etc.):	Room temperature
Administration Instructions:	See other application sections
Possible Untoward Effects, Their Symptoms & Treatment:	See other application sections
Potential or Actual Antidotes for Excessive or Adverse Drug Effect:	N/A
Contraindications and Interactions, If Known:	See other application sections
Investigators Authorized to Prescribe:	

18.4 Reporting Requirements for FDA-regulated research under IND and IDE:

Describe the process for complying with FDA regulatory requirements for adverse event reporting and adverse device effects reporting to the sponsor

Pursuant to 21 CFR 312.2 (Reference a) we have requested the clinical investigation of these drug products (all lawfully marketed) be exempted from the requirement for the study to be done under IND for the following reasons.

1. The investigation is not intended to be reported to FDA as a well-controlled study in support of a new indication for use nor intended to be used to support any other significant change in the labeling for the

drug. This study is entirely initiated, funded and sponsored by Department of Defense without involvement of drug manufacturers.

2. The study drugs that are undergoing investigation are lawfully marketed as a prescription drug product or are currently first line therapies for the indication of travelers' diarrhea. The investigation is not intended to support a significant change in the advertising for the product. The intent of this investigation is to develop the evidence-base to support appropriate guidance of these medications for use in a military deployed setting where simplification of travelers' diarrhea management is aimed at increasing utilization and effectiveness of treating military personnel during deployments.
3. The investigation does not involve a route of administration or dosage level or use in a patient population or other factor that significantly increases the risks (or decreases the acceptability of the risks) associated with the use of the drug product. The drugs used in the design of this study have indications and/or are currently first line therapies in treatment of travelers' diarrhea. We believe that these regimen changes do not constitute undue risk to the subjects based on the following rationale.

18.5 Sponsor (organization/institution/company):

☑ N/A

If applicable, provide sponsor contact information:

19.0 Research Registration Requirements

19.1 ClinicalTrials.gov Registration:

○ Registration is not required
○ Registration pending
◉ Registration complete

"NCT" number:

NCT05677282

19.2 Defense Technical Information Center Registration (Optional):

◉ Registration is not required
○ Registration pending
○ Registration complete

20.0 References and Glossary

20.1 References:

1. Steffen R, Hill DR, DuPont HL. Traveler's diarrhea: a clinical review. JAMA. 2015;313(1):71-80.

2. Sanders JW, Putnam SD, Riddle MS, et al. The epidemiology of self-reported diarrhea in operations Iraqi freedom and enduring freedom. Diagn Microbiol Infect Dis. 2004;50(2):89-93.

3. Connor P, Hutley E, Mulcahy HE, Riddle MS. Enteric disease on Operation HERRICK. J R Army Med Corps. 2013;159(3):229-236.

4. Riddle MS, Tribble DR, Cachafiero SP, Putnam SD, Hooper TI. Development of a travelers' diarrhea vaccine for the military: how much is an ounce of prevention really worth? Vaccine. 2008;26(20):2490-2502.

5. Riddle MS, Martin GJ, Murray CK, et al. Management of Acute Diarrheal Illness During Deployment: A Deployment Health Guideline and Expert Panel Report. Mil Med. 2017;182(S2): 34-52.

6. Riddle MS, Connor BA, Beeching NJ, et al. Guidelines for the prevention and treatment of travelers' diarrhea: a graded expert panel report. J Travel Med. 2017;24(suppl_1):S57-S74.

7. Riddle MS, Connor P, Fraser J, et al. Trial Evaluating Ambulatory Therapy of Travelers' Diarrhea (TrEAT TD) Study: A Randomized Controlled Trial Comparing 3 Single-Dose Antibiotic Regimens With Loperamide. Clin Infect Dis. 2017;65(12):2008-2017.

8. Kantele A, Mero S, Kirveskari J, Lääveri T. Increased Risk for ESBL-Producing Bacteria from Co-administration of Loperamide and Antimicrobial Drugs for Travelers' Diarrhea. Emerg Infect Dis. 2016;22(1):117-120. doi:10.3201/eid2201.151272

9. Chong PP, Chin VK, Looi CY, Wong WF, Madhavan P, Yong VC. The Microbiome and Irritable Bowel Syndrome - A Review on the Pathophysiology, Current Research and Future Therapy [published correction appears in Front Microbiol. 2019 Aug 13;10:1870]. Front Microbiol. 2019;10:1136. Published 2019 Jun 10. doi:10.3389/fmicb.2019.01136

10. Beatty JK, Bhargava A, Buret AG. Post-infectious irritable bowel syndrome: mechanistic insights into chronic disturbances following enteric infection. World J Gastroenterol. 2014;20 (14):3976-3985. doi:10.3748/wjg.v20.i14.3976

11. Garey, Kevin W., et al. "Rifaximin in treatment of recurrent Clostridium difficile-associated diarrhea: an uncontrolled pilot study." Journal of clinical gastroenterology 43.1 (2009): 91-92.

12. Sauvet F, Lebeau C, Foucher S, Flusain O, Jouanin JC, Debonne JM. Operational impact of health problems observed during a four-month military deployment in Ivory Coast. Mil Med. 2009;174(9):921-928. doi:10.7205/milmed-d-05-1008

13. Riddle, Mark S., et al. "Incidence, etiology, and impact of diarrhea among long-term travelers (US military and similar populations): a systematic review." The American journal of tropical medicine and hygiene 74.5 (2006): 891-900.

14. Jiang ZD, Ke S, Palazzini E, Riopel L, Dupont H. In vitro activity and fecal concentration of rifaximin after oral administration. Antimicrob Agents Chemother. 2000;44(8):2205-2206. doi: 10.1128/aac.44.8.2205-2206.2000

15. Frickmann H, Hagen RM, Geiselbrechtinger F, Hoysal N. Infectious diseases during the European Union training mission Mali (EUTM MLI) - a four-year experience. Mil Med Res. 2018 May 31;5(1):19. doi: 10.1186/s40779-018-0166-5.

16. Frickmann H, Warnke P, Frey C, et al. Surveillance of Food- and Smear-Transmitted Pathogens in European Soldiers with Diarrhea on Deployment in the Tropics: Experience from the European Union Training Mission (EUTM) Mali. Biomed Res Int. 2015;2015:573904. doi: 10.1155/2015/573904. Epub 2015 Oct 11.

20.2 Abbreviations and Acronyms:

#	Alpha
AE	Adverse Event
AFRIMS	Armed Forces Research Institute of Medical Science
ANOVA	Analysis of Variance
AMR	antimicrobial resistance
AR	Adverse Reaction
ASCA	Anti-*Saccharomyces cerevisiae* antibody
BATUK	British Army Training Unit Kenya

CBir	Cbir1 flagellin
CLDJ	Camp Lemonnier, Djibouti
CMT	Combat Medical Technician
CPG	TD clinical practice guidelines
CRADA	Cooperative Research and Development Agreement
CRC	Clinical Research Coordinator
CRF	Case Report Form
CRP	C-reactive Protein
CWU	container working unit
DCC	Data Coordination Center
DHA	Defense Health Agency
DoD	Department of Defense
DSA	Data Sharing Agreement
DSMB	Data Safety Monitoring Board
EAEC	enteroaggregative *Escherichia Coli*
EIEC	enteroinvasive *Escherichia coli*
ELISA	Enzyme-linked Immunosorbent Assay
EMF	Expeditionary Medical Facility
EPEC	enteropathogenic *Escherichia coli*
ESBL	Extended-spectrum beta-lactamase
ESBL-PE	Extended-spectrum beta-lactamase-producing Enterobacteriaceae
ETEC	Enterotoxigenic *Escherichia Coli*
FBD	Functional Bowel Disorder
FDA	Food and Drug Administration
FGID	Functional Gastrointestinal Disorders
GI	gastrointestinal
HE	Hepatic encephalopathy
HIPAA	Health Insurance Portability and Accountability Act
HRPO	Human Research Protections Official
IBS	Irritable bowel syndrome
ID	Identification Number
IDCRP	Infectious Disease Clinical Research Program
IL	Interleukin
INR	international normalized ratio
IRB	Institutional Review Board
LLNL	Lawrence Livermore National Laboratory
LMOP	Laboratory Manual of Procedures
LT	heat-labile toxin
MDR	Military Health System Data Repository
MODREC	Ministry of Defence Research Ethics Committee
NAMRU	Naval Medical Research Unit
NHS	UK National Health System
NMCP	Naval Medical Center Portsmouth
NMRC	Naval Medical Research Center
Omp-C	E. coli outer-membrane porin C marker
pANCA	Peri-nuclear Anti-Neutrophil Cytoplasmic Antibodies
PCR	Polymerase Chain Reaction

PE	Paired Ends
PI	Principal Investigator
PP	Per-Protocol Analysis
QA	Quality Assurance
QC	Quality Control
RCT	randomized controlled trial
REDCap	Research Electronic Data Capture
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SAR	Suspected Adverse Reaction
SIBO	small intestinal bowel overgrowth
SOMAmers	Slow Off-rate Modified Aptamers
SOP	Standard Operating Procedure
ST	heat-stable toxin
STEC	shiga-toxin producing *Escherichia coli*
SUSAR	Serious Unexpected Suspected Adverse Reaction
TAC	TaqMan Array Card
TD	Travelers' diarrhea
TID	times daily
TLUS	Time to Last Unformed Stool
TrEAT TD	Trial Evaluating Ambulatory Therapy of Travelers' Diarrhea
TSH	Thyroid Stimulating Hormone
tTg	Anti-Tissue Transglutaminase
UK	United Kingdom
UP	Unanticipated Problem
UP non-AE	Unanticipated problem that is not an Adverse Event
US	United States
USC	United States Code
USU	Uniformed Services University of the Health Sciences