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TITLE:

Dynamics of Gut Microbiota-Pathogen Interactions and Acquisition of Antibiotic Resistance During Travel to High Infectious Burden Regions

PRINCIPAL INVESTIGATOR: Gautam Dantas

CONTRACTING ORGANIZATION: Washington University

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Fort Detrick, Maryland 21702-5012

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4. TITLE AND SUBTITLE

Dynamics of gut microbiota-pathogen interactions and acquisition of antibiotic resistance during travel to high infectious burden regions

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6. AUTHOR(S)

Dantas, Gautam

E-Mail:

5d. PROJECT NUMBER

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5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Washington University, Campus Box 8510, 660 S. Euclid, St. Louis, MO 63110-1010
Naval Medical Research Center, 503 Robert Grant Avenue, Silver Spring, MD 20910-7500

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

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Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

The abstract in Block 14 must be a factual summary stating the purpose, scope, major findings and be an up-to-date report of the progress in terms of results and significance.

Multi-drug resistant (MDR) infections and infectious diarrhea pose high risks to deployed military personnel and travelling civilians. To better understand, predict, and counter the AR threat to deployed military personnel, we are interrogating a longitudinal study of diarrhea in international travelers (both civilian and military) to understand and predict gut microbiota-pathogen interactions and subsequent carriage of MDR organisms (MDROs) following international travel to regions with high infectious disease burdens. In the first phase of the project, we analyzed fecal samples from 156 civilian travelers to Cusco, of whom 113 experienced traveler's diarrhea. We identified MDR E. coli in 34% of diarrheal samples and 24% of asymptomatic samples. We found no obvious changes in the microbial diversity over time while traveling. However, by computing the Bray-Curtis dissimalarity between consecutive samples supplied by the same individual, we found that foreign travel immediately leads to a robust difference between baseline samples, and travelers with diarrhea had substantially more variation in microbiome structure than healthy travelers. By comparing the inter-individual (between consecutive samples from the same individual) versus the inter-individual (comparing samples between individuals) we developed a microbiome perturbation index, and identified microbiome shifts from healthy and diarrheal states. A hallmark of the diarrheal state was an increased ratio of bacteroidetes to firmicutes, corroborating dysbiosis during diarrhea. After shotgun metagenomic sequencing, we identified that in a linear mxed effects model, several species enriched and depleted during diarrhea. E. coli and Dorea formicigenerans were most enriched during diarrhea, and Ruminococcus bromii and Eubacterium rectale were most decreased in diarrheal samples. We developed a computational model that was able to predict sample type with 84% accuracy and identified numerous species that helped classify diarrhea. By utilizing a computational pipeline we identified more unique antibiotic resistance genes and increased abundance of beta-lactamases, efflux pumps, and aminoglycoside resistance genes in diarrheal samples. Taken together, we have demonstrated important features indicative of traveler's diarrhea as well important taxa and resistance genes that can be used as potential biomarkers for traveler's diarrhea.

15. SUBJECT TERMS

Traveler's diarrhea, microbiome, antibiotic resistance gene, E. coli, multi-drug resistant organism, shotgun metagenomics.

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRDC
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1. INTRODUCTION:

Deployed military personnel and travelling civilians are at high risk for multi-drug resistant (MDR) infections and infectious diarrhea. Both MDR infections and infectious diarrhea negatively affect troop combat readiness during deployment. Broad-spectrum antibiotics remain the current standard of care for both treatment and prophylactic prevention of infectious diarrhea. However, antibiotic use can result in dysbiosis of the human gut microbiota, which can negatively affect long-term health, and increase carriage of MDR organisms (MDROs), which can complicate future infections and be transmitted to U.S. healthcare facilities upon return. We will leverage longitudinal studies of diarrhea in international travelers to better understand, predict, and counter gut microbiota-pathogen interactions and subsequent carriage of MDROs during travel to regions with high infectious disease burdens. We will then investigate the effect of specific antibiotic therapies on gut microbiota and antibiotic resistome development in military personnel presenting with diarrhea in two complementary clinical trials. Through this work we will develop predictive computational models which will 1) enable focus of resources toward diarrhea-susceptible personnel, and 2) inform treatments to reduce the risk factors for diarrhea and AR gene transmission, ultimately reducing lost days and costs due to mission-limiting diarrheal and MDR infections.

2. KEYWORDS:

Traveler's diarrhea, microbiome, antibiotic resistance gene, E. coli, multi-drug resistant organism, shotgun metagenomics.

3. ACCOMPLISHMENTS:

What were the major goals of the project?

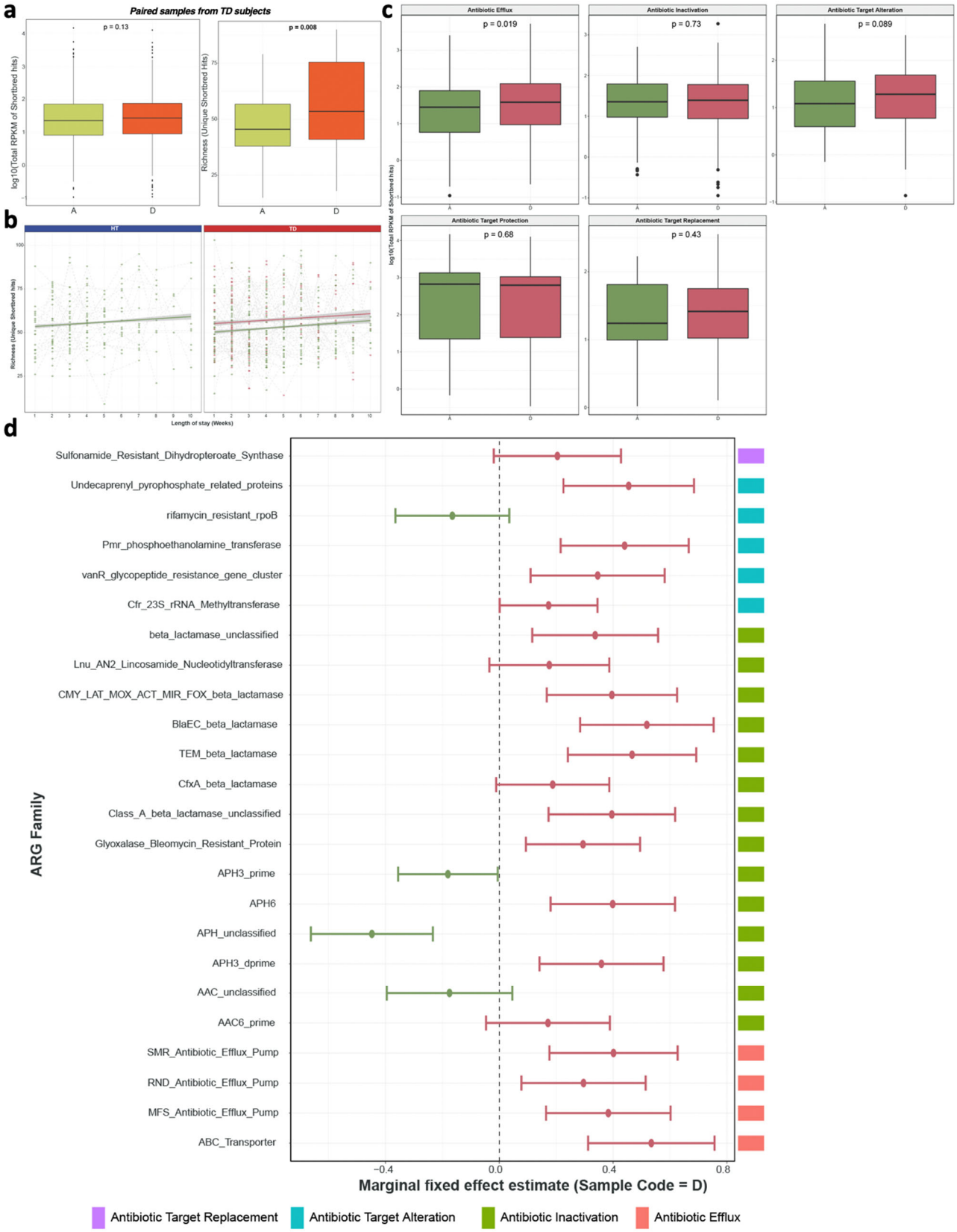
- **Specific Aim 1. Define the process of acquisition, persistence, and transmission of antibiotic resistance (AR) genes during international travel to high infectious burden regions.**
    - Task 1.1. Complement existing AR gene databases by incorporating functionally identified genes from international travelers (Target = 6 mo; Completed = 6 mo).
    - Task 1.2. Assess quantitative changes in the gut resistome of travelers over time (Target = 9 mo; Completed = 9 mo)
    - Task 1.3. Assess differences in the resistance profile of diarrheagenic *E. coli* isolates between symptomatic and asymptomatic patients (Target = 12 mo; Comleted = 12 mo).
    - Task 1.4. Measure the rate at which AR genes are acquired and determine variables responsible for their retention during international travel (Target = 18 mo; 50% complete).
- **Specific Aim 2. Understand host microbiota-pathogen dynamics during international travel to high infectious burden regions.**
    - Task 2.1. Characterize gut microbial communities and assess temporal stability of the gut community structure in response to travel and diarrheal infection (Target = 20 mo).
    - Task 2.2. Identify and characterize novel potential diarrheagenic pathogens in specimens with unknown etiology (Target = 24 mo).
    - Task 2.3. Characterize bacterial isolates in healthy travelers (HT) and traveler's diarrhea (TD) groups, identify virulence factors, and estimate abundance of virulence factors using quantitative PCR (qPCR) (Target = 26 mo).
    - Task 2.4. Develop a computational model to identify biomarkers (discriminatory taxa) that can predict disease severity and MDRO colonization in travelers (Target = 30 mo).
- **Specific Aim 3. Quantitatively analyze the long-term effects of antibiotic therapy on the gut microbiota and resistome in military personnel.**
    - Task 3.1. Characterize long-term changes to the gut microbiota of military personnel resulting from antimicrobial treatment (Target = 36 mo).
    - Task 3.2. Test the ability of various discriminatory features to predict the effect of antimicrobial therapy on the gut microbiota of military personnel (Target = 36 mo).

What was accomplished under these goals?

- **Specific Aim 1. Define the process of acquisition, persistence, and transmission of antibiotic resistance (AR) genes during international travel to high infectious burden regions.**
    - Task 1.1. Complement existing AR gene databases by incorporating functionally identified genes from international travelers (Target = 6 mo; Completed = 6 mo).
        - To determine the antibiotic resistance genes contained in the fecal samples, we made 25 functional metagenomic libraries, comprised of 10 samples each from 250 individuals.

These libraries were screened against 18 antibiotics, and inserts from transformants that survive antibiotic selection were PCR amplified, sheared, barcoded, and pooled for sequencing library platform. These were then sequenced using the Illumina NextSeq platform, and the Illumina paired-end reads were demultiplexed, quality filtered, and the assembly of each selection was performed using our computational pipeline (PARFuMS).

- Task 1.2. Assess quantitative changes in the gut resistome of travelers over time (Target = 9 mo; Completed = 9 mo).
  - To determine AR gene composition and abundance, we obtained metagenomic DNA from 667 stool samples via phenol/chloroform extraction. Whole metagenomic sequencing libraries of these extracts were prepared using a modified Nextera tagmentation protocol and then sequenced on the Illumina NextSeq platform. The de-multiplexed Illumina-paired end reads from each sample were then quality filtered and masked for human DNA contaminants.
  - We first performed a standard BLAST based search against the Comprehensive Antibiotic Resistance Database (CARD) and the ResFinder database to identify >99% similarity to known AR genes. Additionally, we used our in-house curated resistance database Resfams to identify remote homolog AR genes in metagenomes that were not identified by high-identity BLAST to AR databases. These AR genes were then quantified in each metagenome using ShortBRED. Briefly, a set of 1339 unique markers were generated from 2208 antibiotic resistance protein sequences present in CARD. After quality control and manual curation, these unique AR marker lists were used to measure the relative abundance in all 667 sequenced metagenomes.
  - Diarrheal samples were enriched with unique resistance genes, compared to asymptomatic samples (**Fig. 1a,b**). Diarrheal samples had an increased abundance of beta-lactamases, efflux pumps, and aminoglycoside resistance (**Fig. 1c,d**).



- **Figure 1.** Diarrheal samples are enriched in unique resistance genes, including beta-lactamases, efflux pumps, and aminoglycoside resistance. A-B) Shortbred analysis of total number of resistance genes (A) and number of unique ARGs (B) for asymptomatic individuals (marked A) and those with traveler's diarrhea (marked B). C) ARGs were classified by mechanism and are plotted in boxplot. D) Computational model demonstrating ARG families enriched in samples with diarrhea appear to the right of the vertical dotted line and those enriched in asymptomatic samples to the left. Colored panel indicates ARG family.

- Task 1.3. Assess differences in the resistance profile of diarrheagenic *E. coli* isolates between symptomatic and asymptomatic patients (Target = 12 mo; Completed = 12 mo).

- Diarrheagenic *E. coli* were identified in the stools of both symptomatic and asymptomatic individuals (Table 1).

Table 1: Prevalence of diarrhea-causing organisms

Organisms	Diarrhea (%)	Asymptomatic (%)	Total (%)
Diarheagenic *E.coli* (DEC)	60 (34.3)	163 (23.7)	223 (25.8)
DAEC	6 (3.4)	29 (4.2)	35 (4.1)
EAEC	9 (5.1)	42 (6.1)	51 (5.9)
EPEC	16 (9.1)	44 (6.4)	60 (7)
EIEC	0 (0)	5 (0.7)	5 (0.6)
ETEC	22 (12.6)	32 (4.7)	54 (6.3)
STEC	7 (4)	18 (2.6)	25 (2.9)
Other Bacteria	**15 (8.6)**	**8 (1.2)**	**23 (2.7)**
Campylobacter spp.	13 (7.4)	6 (0.9)	19 (2.2)
Shigella spp.	2 (1.1)	2 (0.3)	4 (0.5)
Viruses	**13 (7.4)**	**19 (2.8)**	**32 (3.7)**
Norovirus GI	1 (0.6)	0 (0)	1 (0.1)
Norovirus GII	12 (6.9)	19 (2.8)	31 (3.6)
Parasites	**23 (13.1)**	**119 (17.3)**	**142 (16.5)**
Blastocystis hominis	22 (12.6)	114 (16.6)	136 (15.8)
Cyclospora cayetanensis	0 (0)	1 (0.1)	1 (0.1)
Giardia spp.	1 (0.6)	4 (0.6)	5 (0.6)
No. of samples in which pathogen detected	**87 (49.7)**	**271 (39.4)**	**358 (41.5)**
No. of samples in which no pathogen detected	**88 (50.3)**	**417 (60.6)**	**505 (58.5)**
Total Samples	**175**	**688**	**863**

- *E. coli* isolates were cultured and screened for resistance against 20 antibiotics (**Table 2**). We found these *E. coli* to be resistant to multiple antibiotics with 68% of them qualifying as multi-drug resistant (greater than 3 antibiotic classes) (**Figure 2**).

Table 2: Resistance pattern of diarrheagenic E. coli *strains*

Antimicrobial Category	Agent	Diarrheagenic *E. coli* (DEC strains)		
		Resistant n (%)	Intermediate n (%)	Susceptible n (%)
Penicillins	Ampicillin (Amp)	141 (62.1)	11 (4.8)	75 (33)
	Ticarcillin (TIC-75)	128 (59.3)	3 (1.4)	85 (39.4)
Penicillin + beta-lactamase inhibitors	Amoxicillin-Clavulanate (AMC)	37 (16.3)	51 (22.5)	139 (61.2)
	Ticarcillin-Clavulanate (TIM-85)	80 (41.9)	11 (5.8)	100 (52.4)
1st generation cephalosporins	Cefalotin (CF)	40 (17.5)	69 (30.3)	119 (52.2)
3rd and 4th generation cephalosporins	Ceftriaxone (CRO)	4 (1.8)	3 (1.3)	221 (96.9)
	Ceftazidime (CAZ)	0	0	191 (100)
	Cefotaxime-clavulanate (CTX CLA)	0	2 (1)	189 (99)
	Cefepime (FEP-30)	3 (1.3)	1 (0.4)	224 (98.2)
Carbapenems	Imipenem (IPM-10)	0	0	228 (100)
Macrolides	Erythromycin (E)	215 (94.3)	13 (5.7)	0
	Azithromycin (AZM)	52 (22.9)	47 (20.7)	128 (56.4)

Folate pathway inhibitors	Trimethoprim-Sulfamethoxazole (SXT)	105 (55.6)	3 (1.6)	81 (42.9)
Floroquinolones	Ciprofloxaxin (CIP)	17 (7.5)	22 (9.6)	189 (82.9)
Chloramphenicol	Chloramphenicol (C3))	22 (9.7)	1 (0.4)	203 (89.8)
Tetracyclines	Tetracycline (TE)	119 (52.2)	1 (0.4)	108 (47.4)
Aminoglycosides	Gentamycin (GM)	8 (3.5)	0	219 (96.5)
	Amikacin (AN-30)	0	0	228 (100)
Nitrofurans	Nitrofurantoin (F/M)	0	0	191 (100)

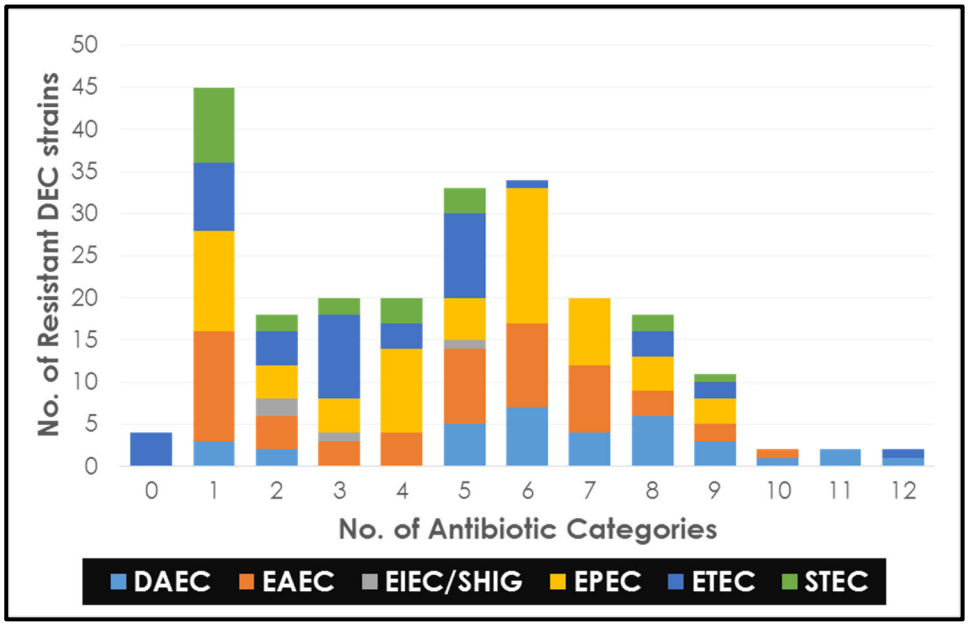


Figure 2. Number of isolates resistant to numbers of antibiotic categories based on culture methods.

- Isolated DNA from 197 *E. coli* isolates from fecal samples that tested positive for diarrheagenic pathogens. Antibiotic resistance gene pattern demonstrated numerous multi-drug resistant pathogens when phenotypically tested.

- **Specific Aim 2. Understand host microbiota-pathogen dynamics during international travel to high infectious burden regions.**
  - Task 2.1. Characterize gut microbial communities and assess temporal stability of the gut community structure in response to travel and diarrheal infection (Target = 20 mo).
    - Gut community dynamics were analyzed in 667 stool samples—which were sequenced on the Illumina NextSeq. We examined the composition of gut microbiota at phylum level in diarrheal samples and asymptomatic samples from same individuals (control) using shotgun metagenomics.
    - There was no significant change in alpha diversity (a measure of diversity within a sample) during international travel and diarrheal episode (**Fig. 3a-c**). However, both the Healthy Traveler and Diarrhea groups had significant divergence of Bray-Curtis dissimilarity (a measure of dissimilarity between samples) baseline (**Fig. 3d**). During the course of stay, the microbiome is temporally stable in both the Healthy Traveler and Diarrhea groups, but there is greater apparent variation within the Diarrhea group. **(Fig. 3e)**.

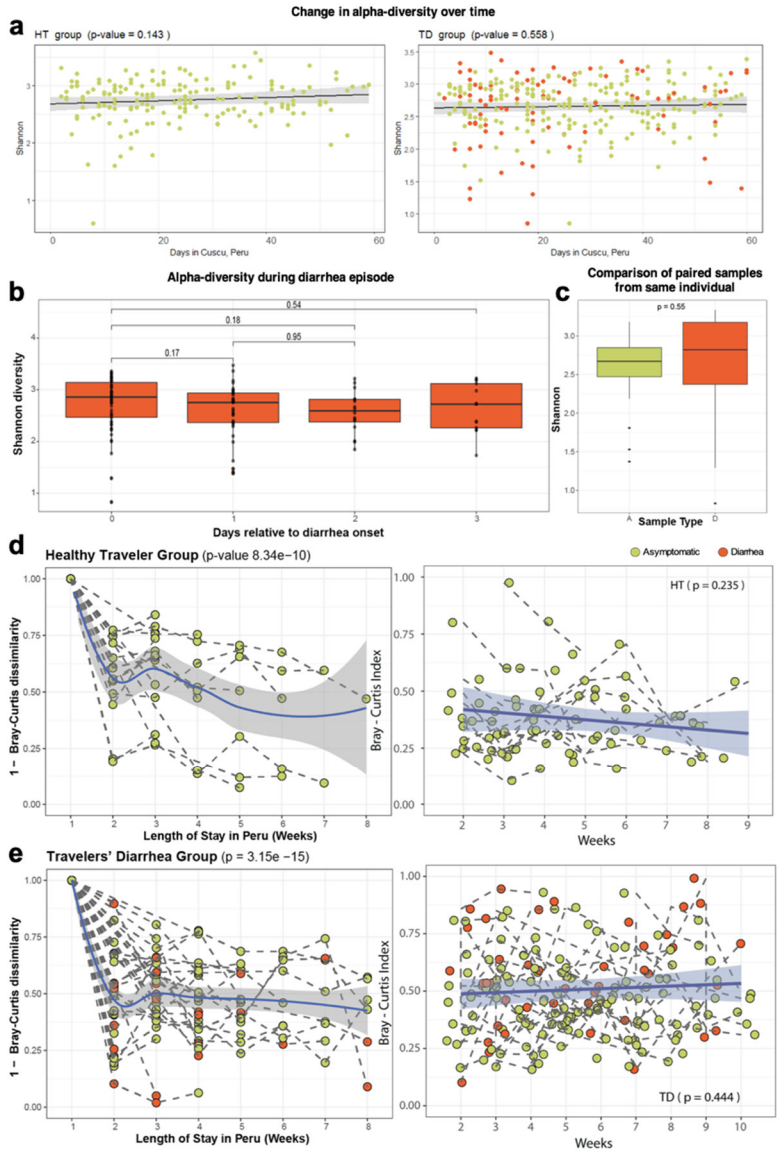


Figure 3. No significant change in alpha diversity during international travel, and diarrheal episode. a) measurement of alpha diversity over time in metagenomic fecal samples between healthy travelers (HT) and those with traveler's diarrhea (TD). b) within TD group, Shannon diversity is shown relative to diarrhea onset. c) for same individual with traveler's diarrhea, Shannon index is displayed for asymptomatic or diarrheal samples. d and e) Bray Curtis index (1-Bray-Curtis dissimilarity) is demonstrated for healthy travelers (d) and those with traveler's diarrhea (e) over time.

- Pairwise comparison showed higher relative abundance of *Bacteroidetes* (p-value: 0.00089) and lower abundance of *Firmicutes* (p-value: 0.0034) in diarrheal samples compared to asymptomatic samples (**Fig 4**). Overall, we observed a signature dysbiotic gut microbiome profile of high Bacteroidetes: Firmicutes ratios (p-value: 0.00042) in the diarrheal samples compared to asymptomatic samples in individuals who had diarrhea during their trip.

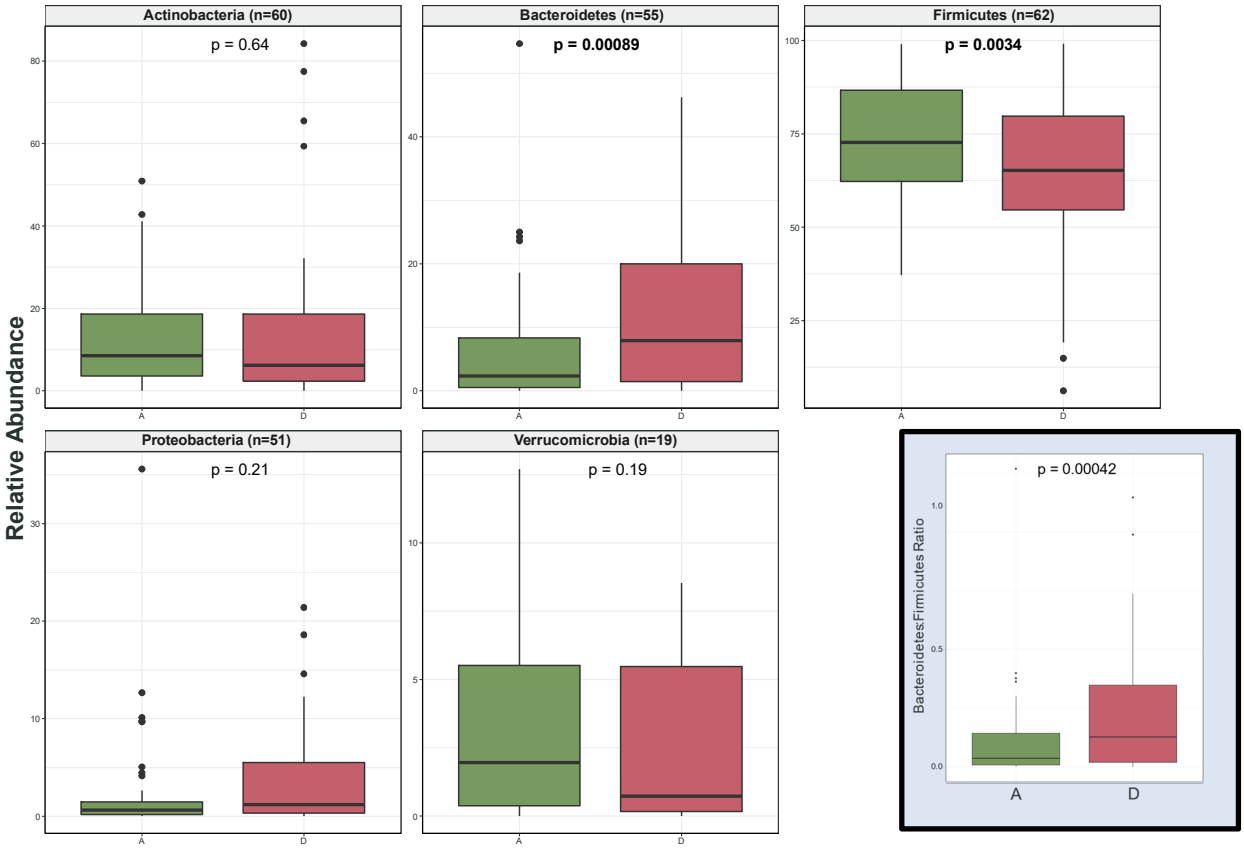


Figure 4. Diarrhea alters gut microbiota composition. Box plot reporting the relative abundance of phyla between paired asymptomatic (A) and diarrheal (D) samples.

- We identified several key species associated with asymptomatic and diarrheal samples. *Escherichia coli* and *Dorea formicigenerans* were the top two species enriched in diarrheal samples, while *Ruminococcus bromii* and *Eubacterium rectale* were the top two depleted (**Fig. 5a,b**)

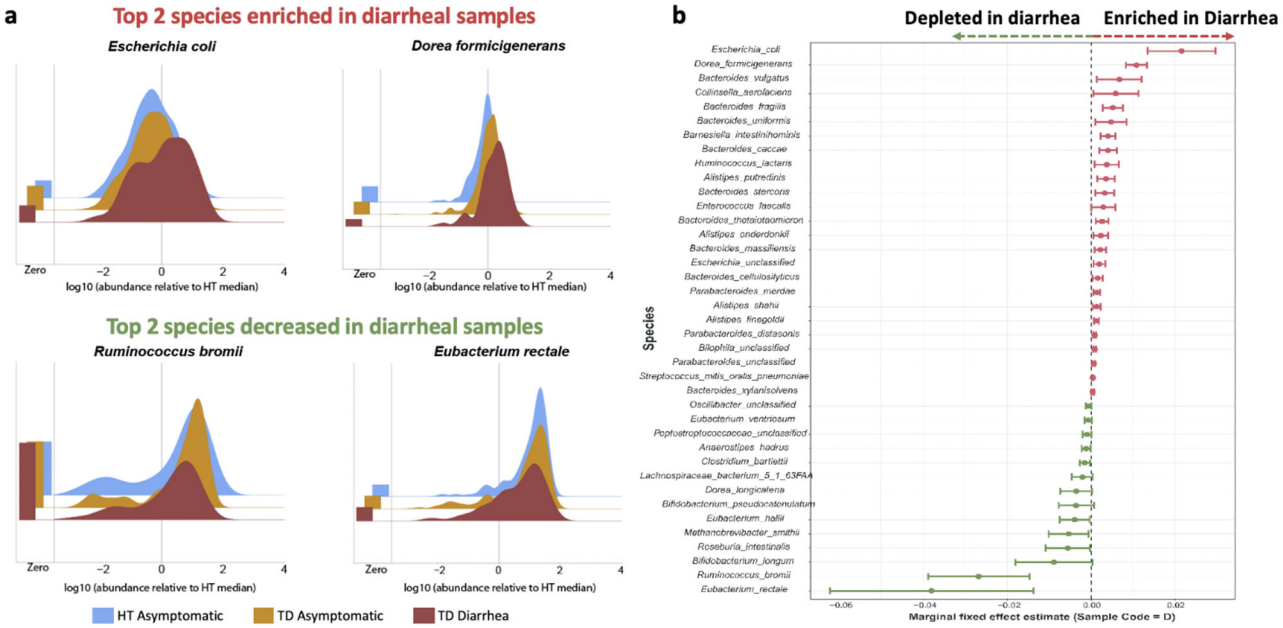


Figure 5. Key species associated with asymptomatic vs. diarrheal samples. a) top 2 species identified by linear mixed models enriched in diarrheal samples or depleted in diarrheal samples. b) full list of species enriched or depleted in diarrheal samples.

- Task 2.2. Identify and characterize novel potential diarrheagenic pathogens in specimens with unknown etiology (Target = 24 mo).

- Task 2.3. Characterize bacterial isolates in healthy travelers (HT) and traveler's diarrhea (TD) groups, identify virulence factors, and estimate abundance of virulence factors using quantitative PCR (qPCR) (Target = 26 mo).
- Task 2.4. Develop a computational model to identify biomarkers (discriminatory taxa) that can predict disease severity and MDRO colonization in travelers (Target = 30 mo).

- **Specific Aim 3. Quantitatively analyze the long-term effects of antibiotic therapy on the gut microbiota and resistome in military personnel.**
  - Task 3.1. Characterize long-term changes to the gut microbiota of military personnel resulting from antimicrobial treatment (Target = 36 mo).
    - The first batch of TrEAT TD samples have arrived at WUSM.
  - Task 3.2. Test the ability of various discriminatory features to predict the effect of antimicrobial therapy on the gut microbiota of military personnel (Target = 36 mo).
  -

What opportunities for training and professional development has the project provided?
Manish Boolchandani, Drew Schwartz, and Kevin Blake are the primary members of the Dantas laboratory working on the project. Gautam Dantas is an expert on microbiome structure and antibiotic resistance gene discovery, transfer, and impact. Manish Boolchandani, Drew Schwartz, and Kevin Blake have been mentored 1-on-1 weekly for an hour throughout the last year. Manish Boolchandani is an expert in computational models and bioinformatics and has mentored Drew Schwartz and Kevin Blake in computational models. In order to gain further expertise in computational modeling Drew Schwartz participated in an R programming course operated by the Washington University Institute for Informatics. Drew Schwartz is an infectious disease physician and has mentored Kevin Blake and Manish Boolchandani in clinically relevant measures of diarrhea and patient outcomes. Furthermore, bi-monthly journal club within the laboratory includes microbiota analysis papers that have collectively increased the knowledge of the primary trainees of this grant.

How were the results disseminated to communities of interest?
Nothing to report

What do you plan to do during the next reporting period to accomplish the goals?
During the next reporting period, we will continue to determine the acquisition of and transfer of antibiotic resistance genes during travel to areas of high infectious burden. We will continue to refine our computational models in this cohort of civilian travelers to Cusco. In the immediate next funding period, we will extract metagenomic DNA and sequence fecal samples from a placebo controlled clinical trial of 2 regimens to treat TD in deployed soldiers.

4. IMPACT:
What was the impact on the development of the principal discipline(s) of the project?
The chief deliverable of our work is an increased understanding of factors altering the gut microbiota during travel and how these factors affect travelers' risk of developing diarrhea. We have characterized the changes accompanying travel to high infectious burden areas as well as the impact of traveler's diarrhea on the microbiota. A key feature of dysbiosis accompanying traveler's diarrhea is an increased Bacteriodetes:Firmicutes ratio. Additionally, we identified species enriched or most depleted in traveler's diarrhea relative to healthy samples. Utilizing computational models we developed a classifier that could identify diarrheal samples with 84% accuracy. These key features helping to distinguish traveler's diarrhea versus asymptomatic samples will be used as a foundation to define hallmarks of microbial dysbiosis during traveler's diarrhea. We further identified unique antibiotic resistance genes present in diarrheal samples indicative of either increased acquisition of environmental resistance genes and/or potential increased dissemination to the environment. These deliverables are directly translatable to the clinic and will provide a solid foundation to inform future treatments limiting and preventing adverse health events that currently compromise U.S. military operations worldwide, including diarrhea and antibiotic resistance.

What was the impact on other disciplines?
Nothing to report

What was the impact on technology transfer?
Nothing to report

What was the impact on society beyond science and technology?
Nothing to report

5. CHANGES/PROBLEMS:
Changes in approach and reasons for change
Nothing to report

Actual or anticipated problems or delays and actions or plans to resolve them
Nothing to report

Changes that had a significant impact on expenditures
Nothing to report

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents
Nothing to report

Significant changes in use or care of human subjects
Nothing to report

Significant changes in use or care of vertebrate animals
Nothing to report

Significant changes in use of biohazards and/or select agents
Nothing to report

6. PRODUCTS:
Publications, conference papers, and presentations

Journal publications.
Nothing to report

Books or other non-periodical, one-time publications.
Nothing to report

Other publications, conference papers, and presentations.

1. Gautam Dantas, “Predicting and combating biotic and abiotic disruptions to diverse microbiomes”. Invited Speaker. CSHL Inaugural Microbiome Conference, Cold Spring Harbor, NY. 18-21 July 2019.
2. Gautam Dantas, “Predicting and combating biotic and abiotic disruptions to diverse microbiomes”. Invited Seminar Speaker and Project Update. Naval Medical Research Center, Silver Spring, MD. 28th August 2019. Included collaboration meeting between Gautam Dantas, PI, and NMRC colleagues Mark Simons, Sandra Isidean, Chad Porter, and Ramiro Gutierrez as well as IDCRP collegues Mark Riddle and David Tribble
3. Gautam Dantas, “Predicting and combating biotic and abiotic disruptions to diverse microbiomes”. Invited Speaker. 2nd Geneva Infection Prevention and Control Think Tank, Geneva, Switzerland. 8-10 September 2019.
4. Gautam Dantas, “Predicting and combating biotic and abiotic disruptions to diverse microbiomes”. Invited Speaker. 5th International Conference of Prevention and Infection Control, Geneva, Switzerland. 10-13 September 2019.
5. Gautam Dantas, “Predicting and combating biotic and abiotic disruptions to diverse microbiomes”. Endowed Chair Seminar Speaker. Snyder Institute, University of Calgary, Calgary, Canada. 18th October 2019.

Website(s) or other Internet site(s)
Nothing to report

Technologies or techniques
Nothing to report

Inventions, patent applications, and/or licenses
Nothing to report

Other Products
Nothing to report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS:

What individuals have worked on the project?

Name: *Dr. Gautam Dantas, PhD*
Project Role: *PI*
Researcher Identifier (e.g. ORCID ID):
Nearest person month worked: *1.80*
Contribution to Project: *Dr. Dantas has worked on coordinating contact between WUSM and NMRC and facilitated obtaining IRB and CRADA approvals. Additionally, he has met with Drew and Manish at least weekly to discuss data interpretation and analysis.*

Name: *Dr. Mark Simons, PhD, D(ABMM)*
Project Role: *PI*
Researcher Identifier (e.g. ORCID ID):
Nearest person month worked: *1.0*
Contribution to Project: *Dr. Simons obtained IRB approval at NMRC. He also coordinated contact with the individuals responsible for sample collection and communicated routinely with WUSM collaborators (Drew Schwartz, Manish Boolchandani, and Gautam Dantas)*

Name: *Sandra Isidean, PhD*
Project Role: *AI*
Researcher Identifier (e.g. ORCID ID):
Nearest person month worked: *1.8*
Contribution to Project: *Dr. Isidean, NMRC, coordinated sample and data exchange from completed IRB approved clinical trials to support the advanced characterization and analysis delineated in the project.*

Name: *Manish Boolchandani*
Project Role: *Graduate student*
Researcher Identifier (e.g. ORCID ID):
Nearest person month worked: *8.25*
Contribution to Project: *Manish has been analyzing temporal resistance gene data among foreign travelers. He has also developed and optimized the computational pipeline for ARG analysis. He has analyzed metagenomic sequencing data for ARG and taxonomy analysis. Additionally, he has met weekly with Dr. Dantas, Dr. Schwartz and Kevin Blake to discuss data.*

Name: *Drew Schwartz, MD, PhD*
Project Role: *Clinical fellow*
Researcher Identifier (e.g. ORCID ID): *3-1568-7733*
Nearest person month worked: *4.0*
Contribution to Project: *Dr. Schwartz has coordinated discussions between NMRC and WUSM. He has assisted Manish and Kevin in data interpretation and analysis and curating metadata during their weekly meetings.*

Name: *Jie Ning*
Project Role: *Research Technician*
Researcher Identifier (e.g. ORCID ID):
Nearest person month worked: *5.25*
Contribution to Project: *Jie extracted DNA from samples and assisted in library preparation of functional metagenomic libraries.*

Name: *Bin Wang*
Project Role: *Research Technician*

Researcher Identifier (e.g. ORCID ID):
Nearest person month worked: *4.6*
Contribution to Project: *Bin assisted Jie and Manish in making sequencing libraries*

Name: *Kevin Blake*
Project Role: *Graduate Student*
Researcher Identifier (e.g. ORCID ID):
Nearest person month worked: *6.0*
Contribution to Project: *Kevin coordinated discussions between NMRC and WUSM. He also discussed interpretation of data with Dr. Dantas, Manish, and Dr. Schwartz.*

Name: *Sanket Patel*
Project Role: *Research Technician*
Researcher Identifier (e.g. ORCID ID):
Nearest person month worked: *8.0*
Contribution to Project: *Sanket assisted Bin, Manish, and Jie in creating sequencing libraries.*

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?
Yes.

What other organizations were involved as partners?
Nothing to Report

8. SPECIAL REPORTING REQUIREMENTS:

QUAD CHARTS: See attached

9. APPENDICES:
None