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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 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:

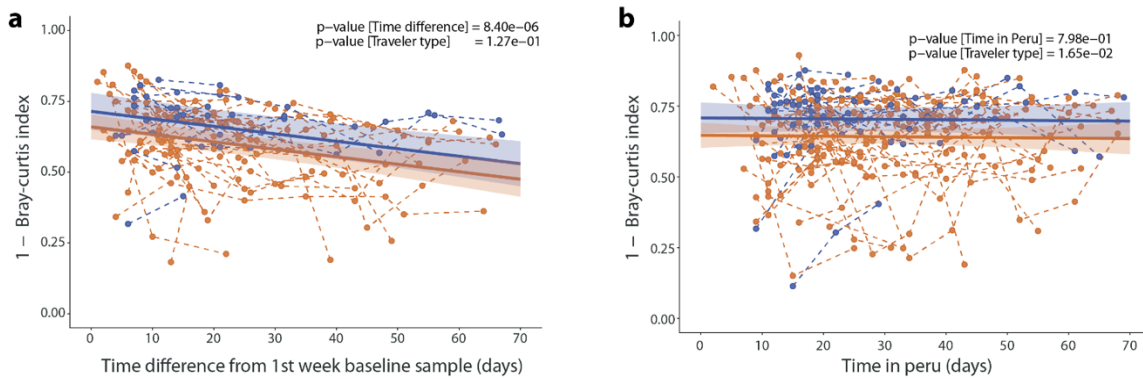
**What were the major goals of the project?**

- **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; Completed = 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; Completed = 18 mo).
- **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; Completed 21 mo).
  - Task 2.2. Identify and characterize novel potential diarrheagenic pathogens in specimens with unknown etiology (Target = 24 mo; 75% Complete).
  - 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; 90% Complete).
  - 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; Completed).
- **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; 20% complete).
  - 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?**

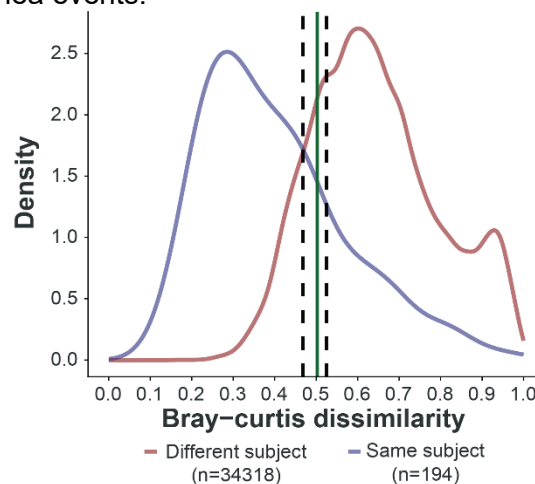
- **Specific Aim 2. Understand host microbiota-pathogen dynamics during international travel to high infectious burden regions.**

- 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; Completed 21 mo).
  - Previously, we reported that taxonomic composition within individuals from both Healthy travelers (HT) and Diarrhea (TD) group diverged significantly from their baseline samples over time (**Fig. 2.1.1a**). These changes were more pronounced in individuals of TD group, where taxonomic profiles of consecutive samples were more dissimilar compared to HT individuals (**Fig. 2.1.1b**).



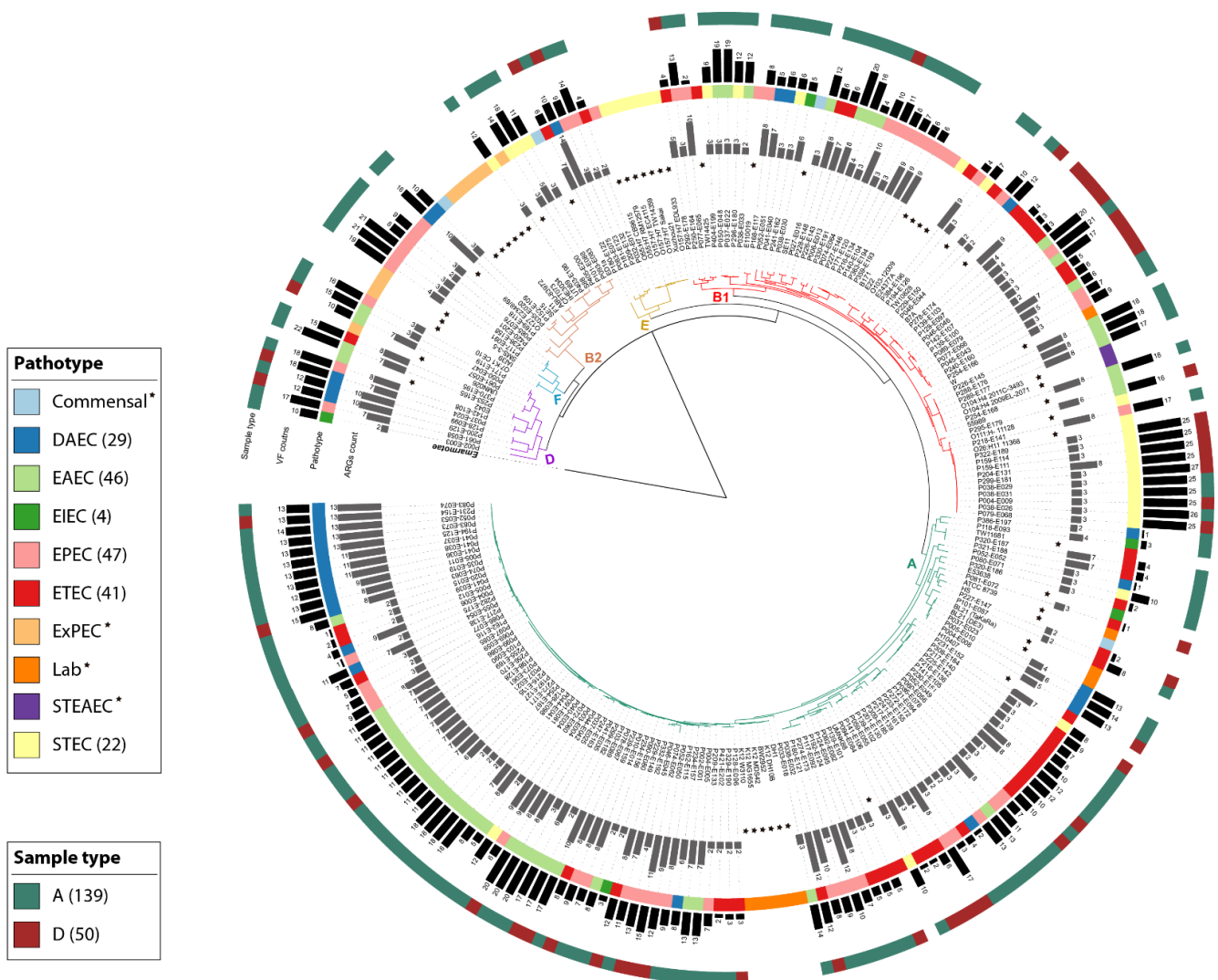
**Figure 2.1.1:** Comparison of Bray-Curtis dissimilarity within individual. (a) Taxonomic divergence from the baseline samples over time for HT (Blue) and TD (Orange) groups (b) Compositional temporal stability measured by comparing consecutive samples in HT (Blue) and TD (Orange) groups. The subjects in the TD group showed lower stability compared to HT group.

- To further investigate the temporal stability, we utilized the fact that the dissimilarity between samples from same individual is less than the dissimilarity between samples from different individuals. Thus, we defined “microbiome shifts” events when the Bray-Curtis dissimilarity between two consecutive time-point of the same person (intra-individual) is greater than the dissimilarity between two different individuals (inter-individual). Using this definition, we calculated the dissimilarity threshold (0.52) by comparing the distribution of Bray-Curtis dissimilarity between the consecutive time points and dissimilarity between samples from different individuals (**Fig. 2.1.2**). We then identified “shift” events where Bray-Curtis dissimilarity between two consecutive timepoints (collected within a week) was greater than the threshold (0.52). We observed 28 of 128 possible shifts (21.9%) in HT individuals and 113 of 279 possible shifts (40.5%) in diarrhea group. The rate of shifts in microbiome was significantly higher during the diarrhea events.



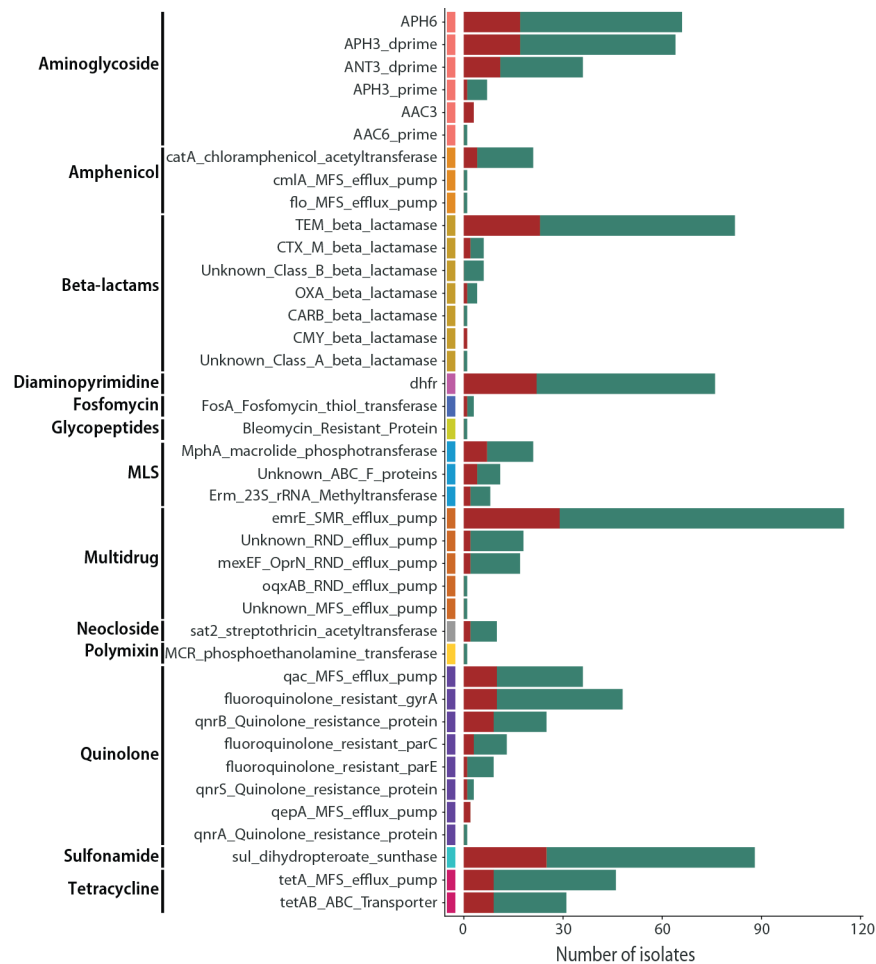
**Figure 2.1.2:** Distribution of Bray-Curtis dissimilarities between samples from the same subject, versus those from different individuals to define ‘microbiome shift’.

- Task 2.2. Identify and characterize novel potential diarrheagenic pathogens in specimens with unknown etiology (Target = 24 mo; 50% Complete)
  - In our initial screening of subset of diarrheal samples, we could not detect causative agents using multiplex PCR and thus, in the grant, we proposed to use Biofire FilmArray GI panel to detect potential diarrheagenic pathogens in our cohort. However, our initial high-resolution metagenomics screening enabled the identification of causative enteropathogens that were missed by the multiplex PCR.
- 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; 90 % Complete).
  - Previously, we performed the phylogenomic analysis using 189 DEC isolates and 40 publicly available *E.coli* genomes of diverse pathotypes by constructing the core genome (2,216 genes, >=95% identity) maximum likelihood phylogenetic tree using Roary and RAxM. The DEC isolates were grouped into 6 distinct clusters (corresponding to phylogroups: A, B1, B2, D, E and F) where the majority of isolates belonged to Clade A (106/189; 56.1%) and Clade B1 (56/189; 29.6%). All other phylogroups (Clade B2: 8/189, Clade D: 11/189, Clade E: 5/189) and Clade F: 3/189) were rare and together accounted for less than 15% (n=27) (**Fig. 2.3.1**). No significant association between phylogroups and isolation source (diarrhea vs non-diarrhea samples) was observed (Fisher's exact, P = 0.3), with the exception of the isolates belonging to Clade B2 and Clade F which were isolated only from non-diarrheal samples.



**Figure 2.3.1:** Phylogenetic tree of 189 *E. coli* isolates are grouped into 5 phylogroups based on core-genes alignment using Roary. These phylogroups are depicted in the inner circle as A (green), B1 (red), B2 (orange), D (purple), E (yellow), F (blue). The reference strains within each phylogroup are marked by asterisk signs. The gray barplot denotes the number of ARGs in each isolate and the inner ring indicates the pathotypes. Strains of same pathotype are coded by same color and color annotation is shown in the top left legend. The outer barplot (in black) shows virulence factor count in each strain, and the outermost ring shows asymptomatic (A:green) and diarrheal (D:red) samples.

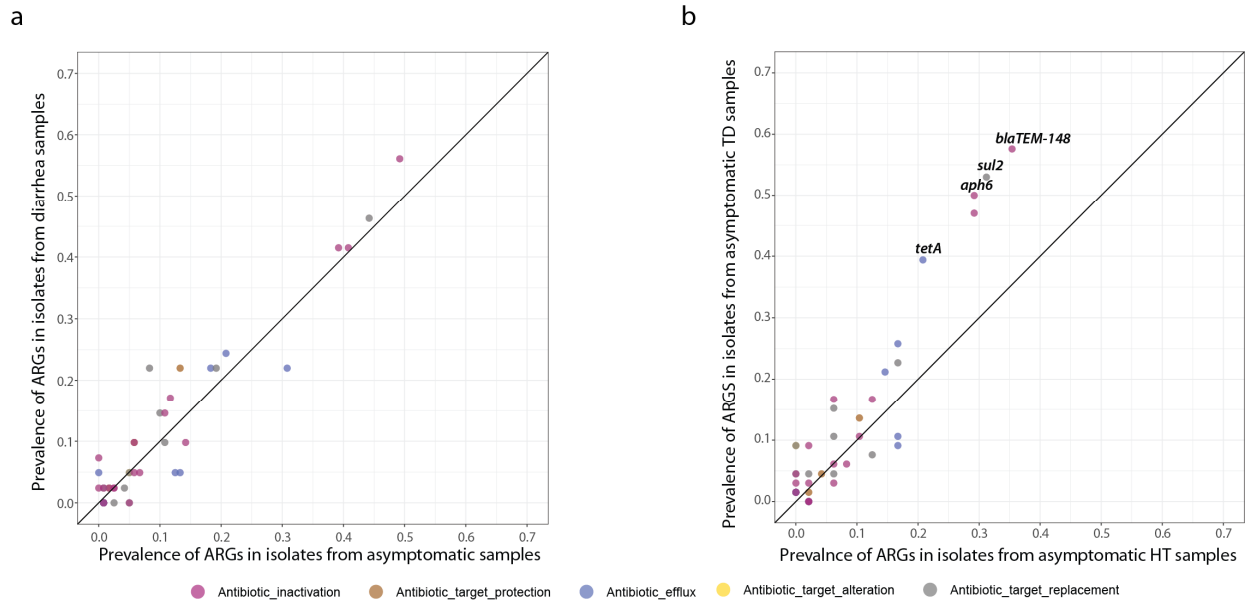
- To further explore the diversity and distribution of AMR determinants harbored by isolates among travelers, we screened the draft genomes for ARGs including genes (*gyrA*, *parC*, *parE*, and *pmrB*) that are known to confer resistance by point mutations. In total, we identified 60 unique AMR determinants, including 21 core (present in  $\geq 95\%$  of isolates) and 39 accessory resistance determinants. The core resistome of DEC isolates include genes that mediate resistance to multiple drugs via efflux pumps whereas the accessory resistomes varied widely among isolates, with no clear association with phylogroups or sample types (diarrhea vs non-diarrhea) (**Fig 2.3.2**).



**Figure 2.3.2:** The frequency of antibiotic resistance identified in the genomes of DEC strains isolated from asymptomatic (green) and diarrheal (red) samples.

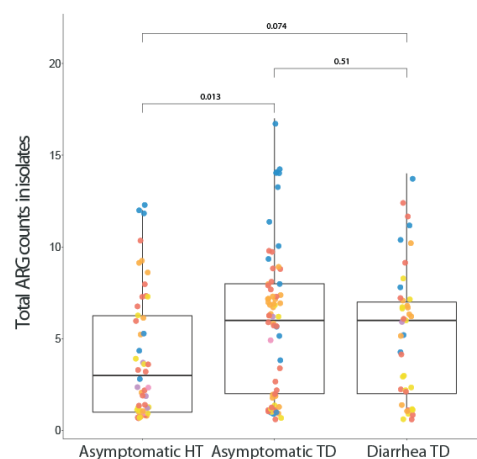
- The most common accessory AMR genotypes identified in our cohort were *emrE* efflux pumps detected in 115 (60.9%) isolates, alleles of *sul* (*sul1*, *sul2*, *sul3*) mediating resistance to sulfonamides in 88 (46.6%) isolates, *bla<sub>TEM</sub>* (*bla<sub>TEM-148</sub>*, *bla<sub>TEM-206</sub>*) in 82 (43.4%) isolates, *dhfr* genes encoding trimethoprim resistance in 76 (40.2%) isolates, and aminoglycoside resistance genes (*aph6-Id*, *aph3*) detected in 66 (34.9%) isolates. In addition, we detected point mutations in *gyrA* (S83L: 30, S83A: 14, D87N: 8, D87Y: 4), *parC* (S80I: 9, A56T: 4, E84G: 2, E84V: 2, S57T: 2) and *parE* (I355T: 3, I529L: 2,

L416F: 2, S458A: 2) quinolone resistance determining region (QRDR) genes in 48 (25.4%), 13 (6.9%) and 9 (4.8%) isolates respectively. The frequency of these prevalent accessory AMR genotypes (present in  $\geq 10\%$  of isolates) were similar in DEC strains isolated from asymptomatic and diarrheal samples; however, comparison of ARGs in DEC strains isolated from asymptomatic samples of HT and TD subjects, showed significant enrichment of *bla*<sub>TEM-148</sub>, *sul2*, *aph6*, and *tetA* genes in TD subjects (**Fig. 2.3.3**).



**Figure 2.3.3:** (a) Prevalence of antibiotic resistance genes identified in DEC strains isolated from asymptomatic and diarrheal samples. (b) Prevalence of antibiotic resistance genes identified in DEC strains isolated from asymptomatic samples from HT and TD subjects.

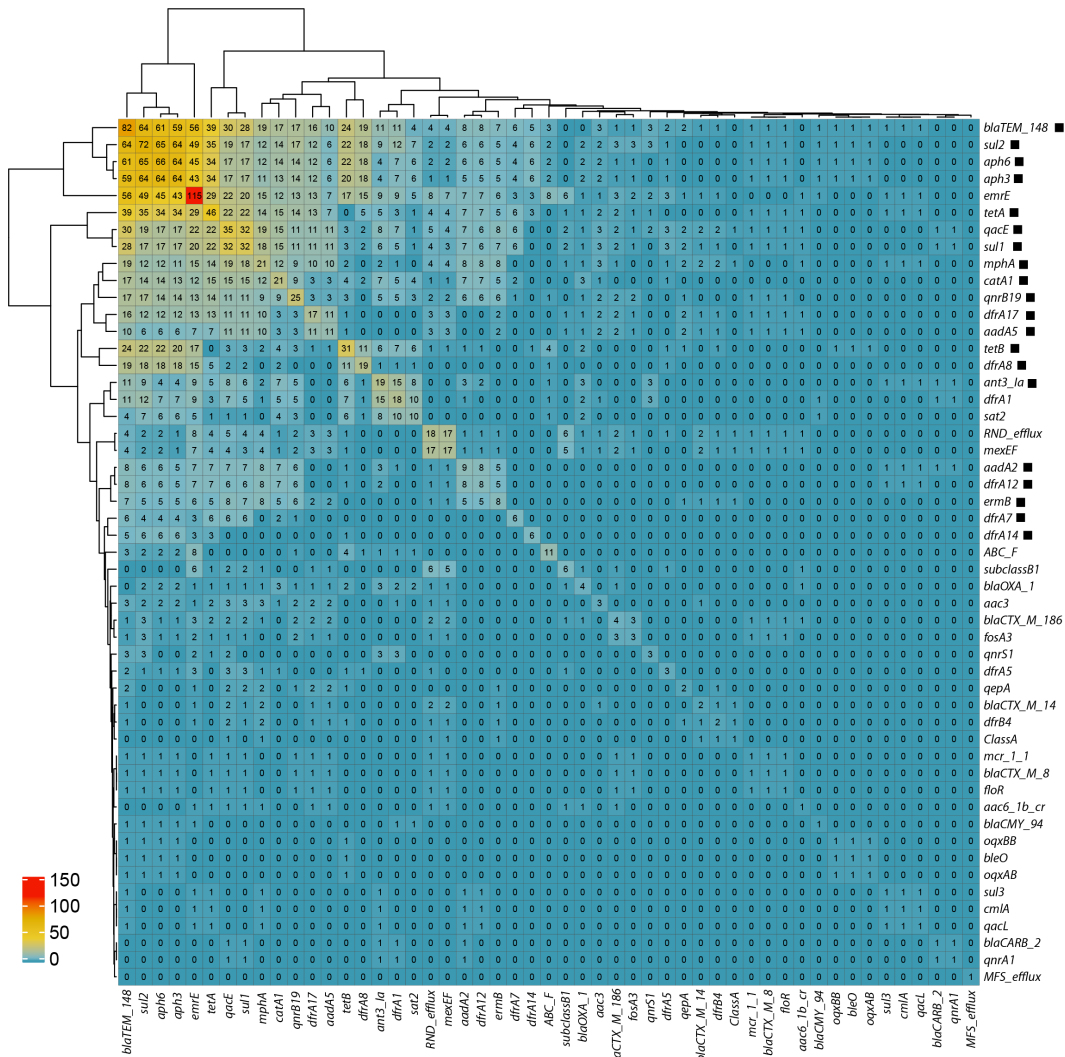
- Interestingly, DEC strains isolated from asymptomatic samples in TD subjects carried more ARGs compared to isolates from HT subjects (non-diarrhea HT vs non-diarrhea TD, p-value=0.01) (**Fig. 2.3.4**) Notably, several DEC strains that were isolated from TD subjects, also carried other MDR genes including, ESBL genes (*bla*<sub>CTX-M</sub>, n=7; *bla*<sub>OXA</sub>, n=3), AmpC (*bla*<sub>CMY</sub>, n=1) gene, and *mcr-1*(n=1) gene.



**Figure 2.3.4:** The boxplot shows the distribution of total unique ARGs carried by the DEC strains extracted from HT and TD subjects. The colored circles represent different DEC strain types.

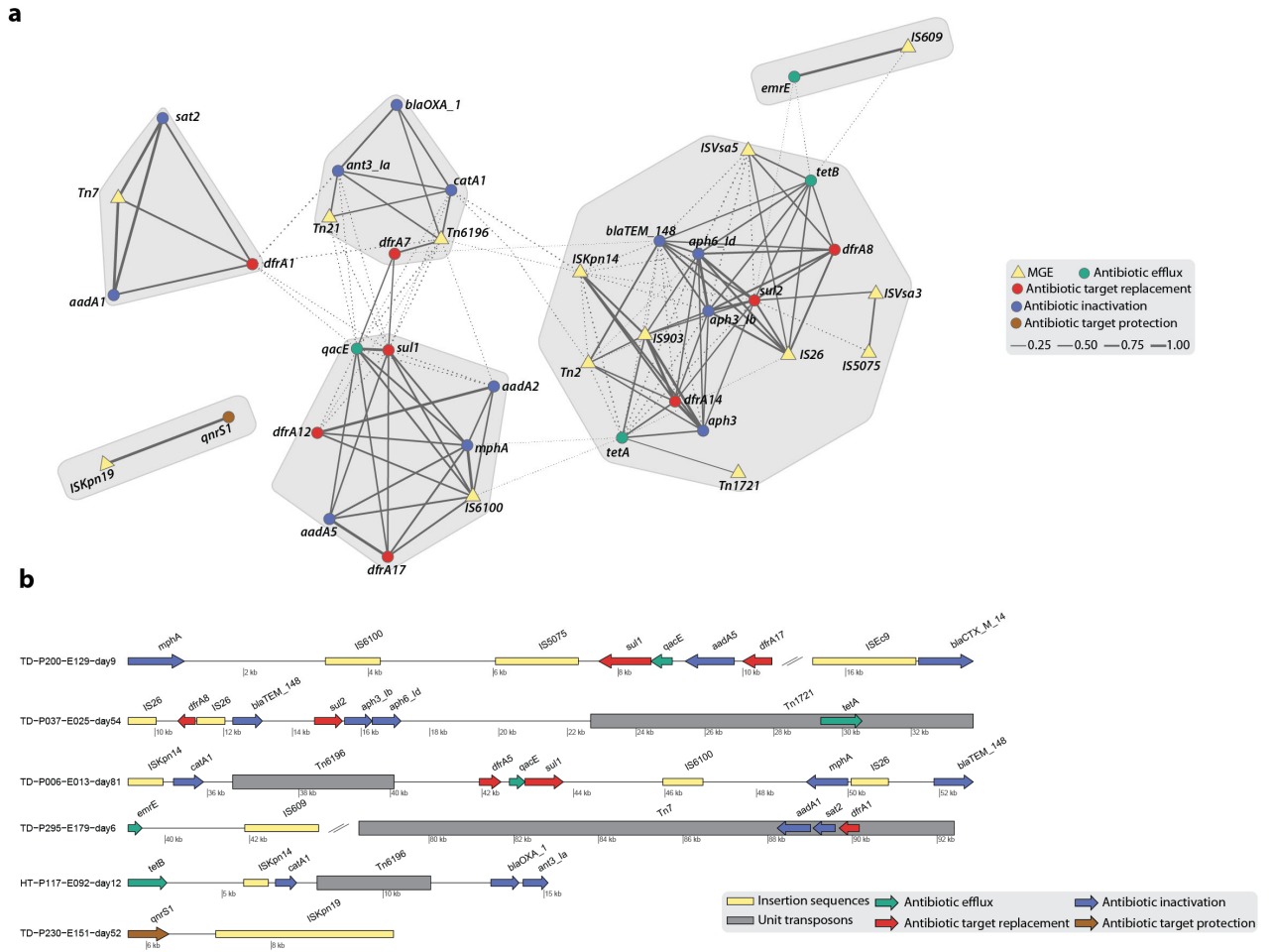
- Pairwise co-occurrence comparison of accessory AMR determinants revealed few highly interconnected group of ARGs (**Fig. 2.3.5**) circulating among DEC isolates. The most common cluster of ARGs (*bla*<sub>TEM-148</sub>, *sul2*, *aph6*, *aph3*, *emrE*, and *tetA*) that were

frequently detected together were also found to be significantly associated with plasmids (49%; 25/51; FDR < 0.05, Fisher's exact test).



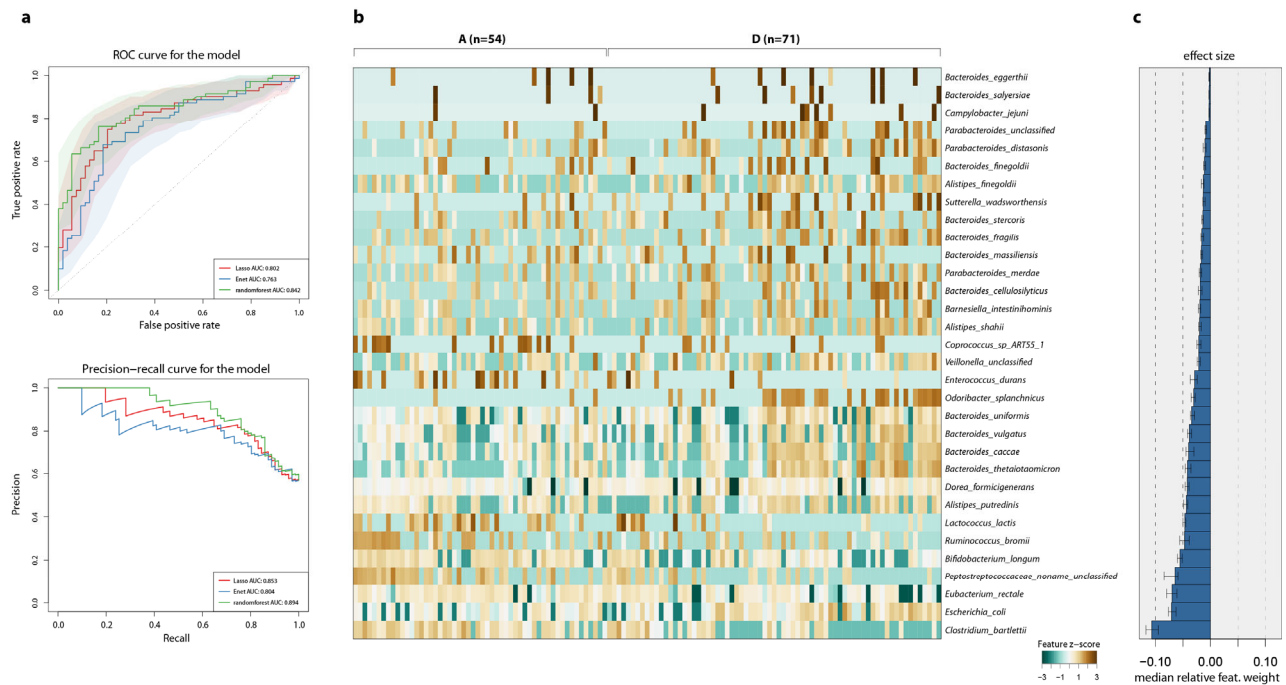
**Figure 2.3.5:** Co-occurrence matrix of accessory ARGs in isolates. The number within each cell represents the total number of isolates that had both the pair of ARGs. ARGs that were significantly associated with plasmid are marked with black squares (■).

- Since these highly prevalent and co-occurring ARGs were frequently found in isolates from TD subjects, we next sought to analyze the genomic context of these ARGs to assess the risk of transmission to other microbes. We identified the instances of mobile genetic elements (MGEs) (unit and composite transposons, insertion sequence) within 5 kb region of the resistance genes using MGEfinder. We then built the co-occurrence network of the observed ARGs with MGEs and identified clusters that were more frequently observed together in the isolates. We identified four major clusters that include ARGs conferring resistance via different mechanism. The genes *sul2*, *aph6*, *aph3* and *bla*<sub>TEM-148</sub> were most commonly occurring group observed in 60 (39.1%) isolates. These genes were often observed in the genetic context of IS26 insertion element (n=12) and Tn2 unit transposon (n=12). We also observed these genes frequently occurring with *dfrA8/dfrA14* genes (n=20) and other mobile elements like IS903 (Fig. 2.3.6).



**Figure 2.3.6:** Co-occurrence network of ARGs and mobile elements among *E.coli* isolates. Nodes represent ARGs (circle) and mobile elements (triangle). The connection between two nodes represented correlation of genes occurring together with mobile elements in the same contig (within 5kb). The solid line represents significant relationships after FDR correction. The shaded regions represents subnetworks b) Examples of arrangement of ARGs and mobile elements identified on the same contig from isolates.

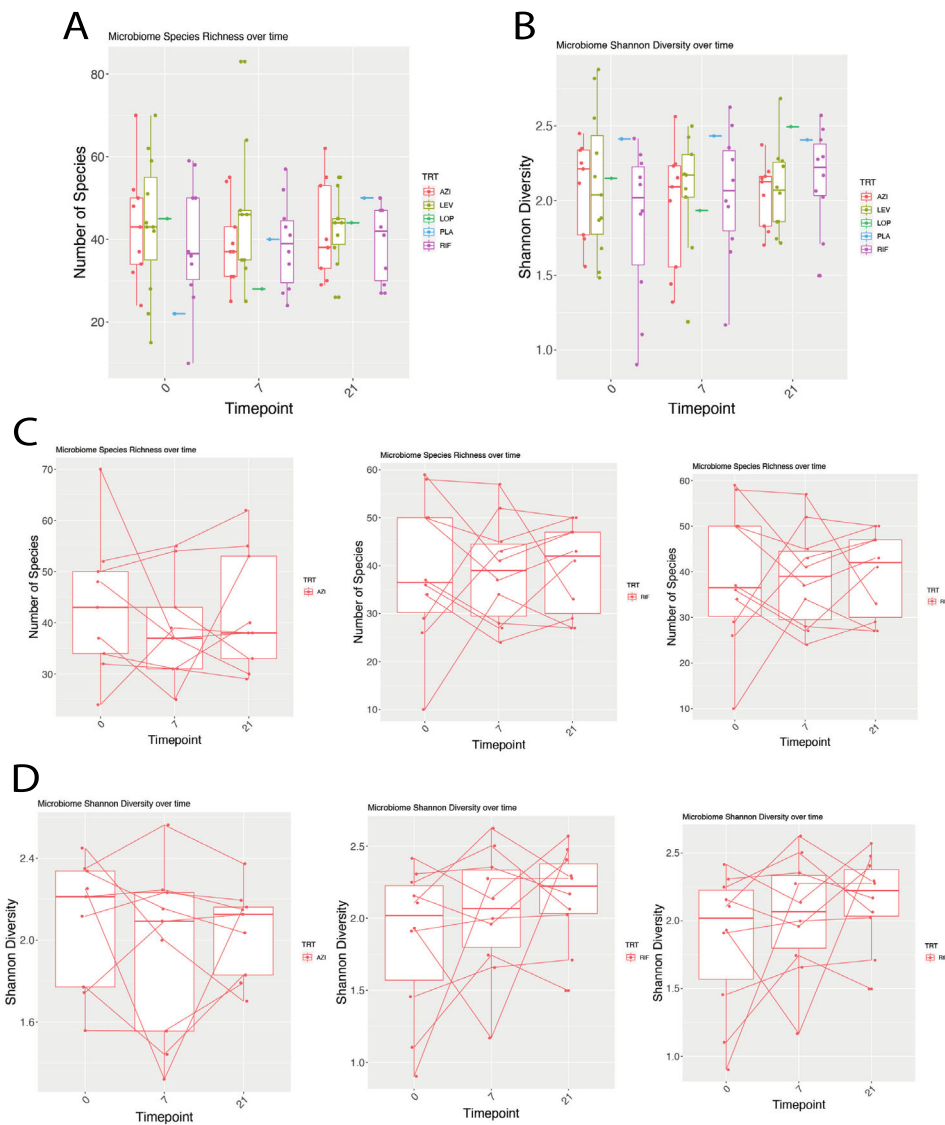
- Task 2.4. Develop a computational model to identify biomarkers (discriminatory taxa) that can predict disease severity and MDRO colonization in travelers (Target = 30 mo; Completed).
  - To further investigate the discriminatory taxa and their ability to distinguish between symptomatic diarrhea and asymptomatic samples, we built machine learning models using Lasso, Enet and Random forest classifier. We built the model using 32 discriminatory taxa that were significantly enriched or depleted in diarrhea compared to asymptomatic samples and achieved the accuracy of up to 90% (Fig. 2.4).



**Figure 2.4:** (a) Receiver operating characteristic (ROC) curve and precision recall curve obtained for the three machine learning models obtained by Lasso, Enet and Random forest. Among these approaches, Random forest performs the best and provides accuracy up to 90%. (b) Thirty-two discriminatory taxa that were used to build the machine learning model. Heatmap shows change in their relative abundance in between A and D samples. (c) Effect size of each species used in the model

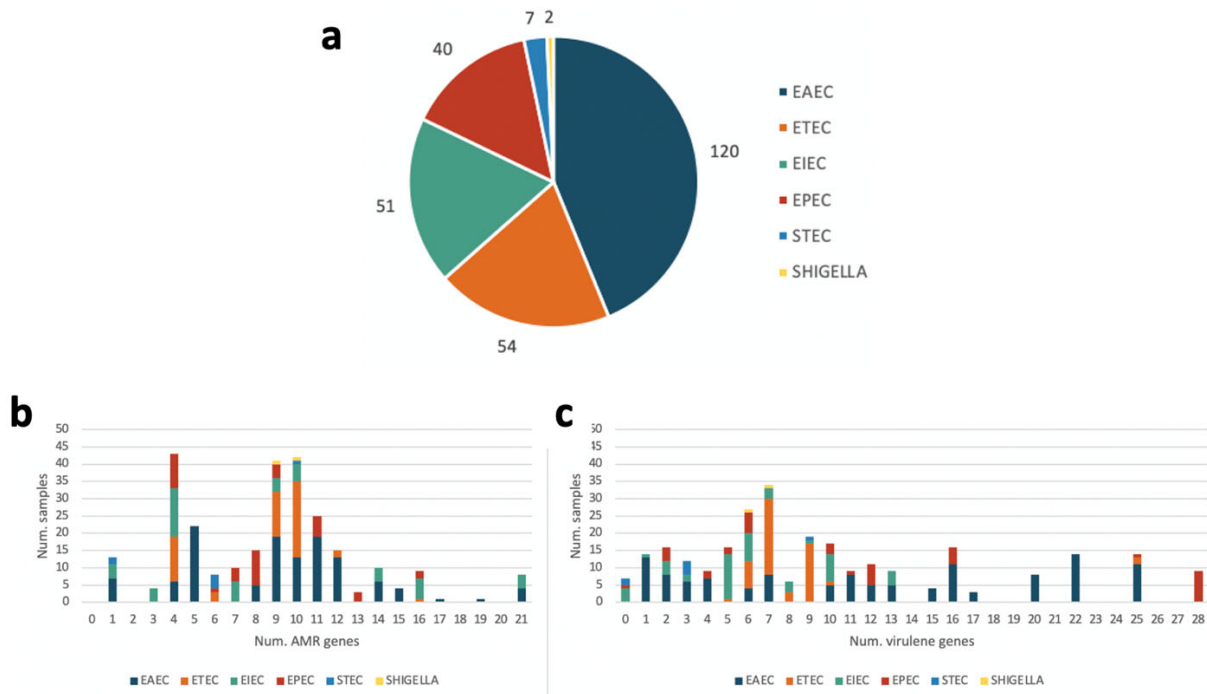
- **Specific Aim 3. Quantitatively analyze the long-term effects of antibiotic therapy on the gut microbiota and resistome in military personnel.**
  - Task 3.1. Characterize long-term changes to the gut microbiota of military personnel resulting from antimicrobial treatment (Target = 36 mo; 20% complete).
    - We have received 200 stool samples from the Treat TD cohort. Included among these were paired (2) samples from the same individual and timepoint, but where one vial from that sample was maintained at -80 °C and the other was involved in a freezer failure which included warming of the samples to above freezing. While many samples involved in the freezer failure had a pair which had not thawed, some samples did not. Therefore, we first wanted to determine whether these samples involved in the freezer failure could still provide useful data, or if they were compromised and needed to be excluded from analyses. To do so, we extracted metagenomic DNA from 43 paired fecal samples (86 total) from 17 individuals where one of the pair was involved in a freezer failure (FF) and the other was never thawed (OK). We then directly compared these paired samples using several metrics to determine what, if any, differences existed between them. Based on these analyses, paired samples demonstrate similar taxonomy (**Fig. 3.1.1a**), are most similar to each other (i.e. do not cluster by freezer status) (**Fig. 3.1.1b**), and do not have significant differences in Shannon diversity (**Fig. 3.1.1c**). Therefore, we conclude that samples involved in a freezer failure and do not have an OK pair are viable for downstream analyses. We will include freezer failure status in all subsequent modeling to ensure that our analyses are not biased by including this information.





**Figure 3.1.2:** Impact of antibiotic therapy on the gut microbiota of military personnel. (A) Number of species present at timepoints 0, 7, and 21 with respect to treatment with azithromycin (AZI), rifampin (RIF), or levofloxacin (LEV) for acute watery diarrhea or azithromycin (PLA) or azithromycin plus loperamide (LOP) for acute febrile diarrhea. (B) Shannon diversity displayed for the same samples as in A. Barplots contain 75% of the range with horizontal line at median. (C) Richness and (D) Shannon diversity over time divided by treatment demonstrating individual microbiome trajectories.

- We have sequenced 293 *E. coli* isolates from the TrEAT TD study. Reads were demultiplexed by barcode and had adapters removed with Trimmomatic. Next, processed reads were assembled into draft genomes using Unicycler, and assembly statistics quantified using Quast and CheckM. 19/293 genomes did not meet quality criteria for downstream analyses ( $\geq 90\%$  completeness,  $<5\%$  contamination,  $<500$  contigs), and will be re-sequenced. The 274 genomes were derived from isolates labelled EAEC (120/274, 43.8%), ETEC (54/274, 19.7%), EIEC (51/274, 18.6%), EPEC (40/274, 14.6%), STEC (7/274, 2.6%), and Shigella (2/274, 0.7%) (**Fig. 3.1.3a**). We next determined these genomes' open reading frames with Prokka, and quantified the number of AMR and virulence genes per genome with amrfinder (**Fig. 3.1.3b-c**).



**Figure 3.1.3:** (a) Pathotypes of 274 assembled TrEAT TD genomes. Quantification of the number of (b) AMR genes and (c) virulence genes by pathotype.

**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 data analysis. Gautam Dantas is an expert on microbiome structure and antibiotic resistance gene discovery, transfer, and impact and computational methods to model these interactions. Manish Boolchandani, Drew Schwartz, and Kevin Blake have been mentored 1-on-1 weekly throughout the last reporting period. Manish Boolchandani and Alaric D’Souza are experts in computational models and bioinformatics and have mentored Drew Schwartz and Kevin Blake in computational models. With the recent lifting of in-lab restrictions, Drew Schwartz, Kevin Blake, Bin Wang, and Jie Ning have processed TreatTD stool samples and *E. coli* isolates for sequencing. Additionally, Manish Boolchandani, Drew Schwartz, Alaric D’Souza and Kevin Blake have continued to participate in weekly analysis meetings. We have also engaged in monthly remote meetings with collaborators at NMRC and IDCRP to discuss issues, analysis, and sample acquisition. As such, Manish Boolchandani, Drew Schwartz, Alaric D’Souza and Kevin Blake are developing skills in computational analysis, working in teams to accomplish a common goal, and analyzing epidemiologic metadata.

**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?**

Because of the continued COVID-19 limitations on laboratory experiments, we will work 80% on analysis and 20% sample processing in the upcoming reporting period. We are currently processing *E. coli* isolates and stool samples from Honduras from the TreatTD cohort for sequencing. We hope to submit the first manuscript describing analysis of the CuscoTD cohort in the next 2-3 months. We will also continue performing preliminary analysis on the first set of samples sequenced from the TreatTD cohort as well as *E. coli* isolates. We anticipate receiving the shipment of stool samples from the PreventTD cohort in the next 1-2 months.

**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. During this reporting period we continued refining computational models that could predict whether a sample was diarrheal or healthy. This computational model can lead to biomarkers of traveler’s diarrhea.

**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**

As indicated in our previous progress reports, we have experienced research delays secondary to the COVID-19 pandemic. Until late-September, sample processing and shipment for the PreventTD study was halted due to laboratory shutdowns. We have continued to advance towards completion of grant milestones by procuring a detailed stool sample inventory and preparing associated metadata for planned analyses. Assuming no further shutdowns, we anticipate receiving 242 paired samples from 121 study participants in the next 2 months. We have also experienced delays in obtaining TrEAT TD stool samples from Kenya (n=245). We are actively working to ensure shipment of these samples from Kenya and hope to do so by early CY2021.

Based on COVID-19 related pandemic delays, we anticipate that we will request a 1 year no cost extension at the end of the original proposed funding period (date) to complete the proposed work.

**Changes that had a significant impact on expenditures**

Actual expenditures (\$893,927) were less than projected expenditures (\$1,147,820). This was primarily driven by an inability to ship, process, and sequence samples due to COVID-19 laboratory partial shutdowns.

**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**

Nothing to report.

**Journal publications.**

Nothing to report.

**Books or other non-periodical, one-time publications.**

Nothing to report.

**Other publications, conference papers, and presentations.**

Nothing to report.

**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): 0000-0003-0455-8370*  
*Nearest person month worked: 1.8*  
*Contribution to Project: Dr. Dantas has worked on coordinating contact between WUSM and NMRC and met with Dr. Schwartz, Kevin, Alaric 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 has coordinated contact with the individuals responsible for sample collection and communicated routinely with WUSM collaborators (Drew Schwartz, Manish Boolchandani, Kevin Blake, and Gautam Dantas).*

*Name: Sandra Isidean, PhD*  
*Project Role: AI*  
*Researcher Identifier (e.g. ORCID ID):*  
*Nearest person month worked: 1.5*  
*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. Dr. Isidean has also coordinated communication between sites as well as ensuring safe transfer of metadata.*

*Name: Manish Boolchandani*  
*Project Role: Graduate student*  
*Researcher Identifier (e.g. ORCID ID): 0000-0001-6038-8957*  
*Nearest person month worked: 9.0*  
*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, Alaric, and Kevin to discuss data. Manish has also participated in monthly collaboration meetings with Dr. Simons and Dr. Isidean.*

*Name: Drew Schwartz, MD, PhD*  
*Project Role: Clinical fellow*  
*Researcher Identifier (e.g. ORCID ID): 0000-0003-1568-7733*  
*Nearest person month worked: 3.9*  
*Contribution to Project: Dr. Schwartz has analyzed TreatTD metagenomes. Dr. Schwartz has also participated in monthly collaboration meetings with Dr. Simons and Dr. Isidean as well as weekly manuscript planning.*

*Name: Jie Ning*  
*Project Role: Research Technician*  
*Researcher Identifier (e.g. ORCID ID):*  
*Nearest person month worked: 4.25*  
*Contribution to Project: Jie extracted DNA from TreatTD fecal samples and assisted Dr. Schwartz and Bin Wang in preparing sequencing libraries.*

Name: Bin Wang  
Project Role: Research Technician  
Researcher Identifier (e.g. ORCID ID):  
Nearest person month worked: 5.04  
Contribution to Project: Bin assisted Jie and Dr. Schwartz in isolating metagenomic DNA and genomic DNA from E. coli isolates and preparing sequencing libraries.

Name: Alaric D'Souza  
Project Role: Graduate student  
Researcher Identifier (e.g. ORCID ID): 0000-0002-8744-8136  
Nearest person month worked: 5.51  
Contribution to Project: Alaric has assisted Manish with analyzing resistance gene data. He has also met weekly with Dr. Schwartz, Kevin Blake, and Dr. Dantas on analytical and computational techniques.

Name: Kevin Blake  
Project Role: Graduate Student  
Researcher Identifier (e.g. ORCID ID): 0000-0003-4247-1209  
Nearest person month worked: 1.50  
Contribution to Project: Kevin coordinated discussions between NMRC and WUSM. He also discussed interpretation of data with Dr. Dantas, Manish, and Dr. Schwartz. Kevin has also participated in monthly collaboration meetings with Dr. Simons and Dr. Isidean.

Name: Robert Thaenert  
Project Role: Postdoctoral Research Associate  
Researcher Identifier (e.g. ORCID ID):  
Nearest person month worked: 2.25  
Contribution to Project: Robert helped with establishment of computational pipelines for genome assemblies from metagenomes.

**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. See attached PCPS reports.

**What other organizations were involved as partners?**

Nothing to report

## **8. SPECIAL REPORTING REQUIREMENTS:**

### **QUAD CHARTS:**

See attached.

## **9. APPENDICES:**

See updated PCPS report for Drs. Dantas and Simons on following pages.

## OTHER SUPPORT DANTAS, GAUTAM

### ACTIVE

#### *No change*

*Title:* Structural, mechanistic, and evolutionary characterization of tetracycline destructases

*Time Commitment:* 1.08 Cal *Performance Period:* 02/11/16 – 01/31/21 *Level of Funding:* Supporting

*Supporting Agency:* NIH/NIAID (Dantas; PI), GMS Tseday Girma

*Goals:* The major goals of this project are to characterize the structure, mechanism and evolutionary profiles of the tetracycline destructases, a novel family of tetracycline inactivating enzymes.

*Aims:* Aim 1: Elucidate

the mechanism of action of the tetracycline destructases. Aim 2: Characterize the evolutionary landscape of the tetracycline destructases. Aim 3: Rationally design and synthesize tetracycline destructase inhibitors.

*Overlap:* No overlap

#### *No change*

*Title:* Washington University & BJC Epi-Center for Prevention of Healthcare Associated Infections

*Time Commitment:* 2.4 Cal *Performance Period:* 07/01/16 – 5/31/21 NCE *Level of Funding:* \$0

*Supporting Agency:* CDC U54CK000162 (Fraser; PI)

*Goals:* The Washington University (WU) and BJC Prevention Epicenter (PE) works collaboratively with CDC and other PEs to perform research and to translate basic, epidemiology and technologic discoveries into new strategies to prevent and decrease healthcare associated infections (HAIs) and antimicrobial resistance (AR). For our core and 3 multicenter collaborative proposals, we organized a team of basic, translational and clinical investigators with expertise in infectious disease, healthcare epidemiology, microbiology, AR, microbiome, and metabolome sciences to work together to perform translational studies to prevent HAIs and AR.

*Aims:* We propose a broad 4-year research strategy in our core proposal focused on developing and testing novel strategies to: document and improve outpatient antibiotic prescribing practices, reduce surgical site infections (SSIs), identify changes in AR and gut microbiome following fecal microbiome transplant (FMT), and identify novel biomarkers to more rapidly diagnose urinary tract infections (UTIs) and avoid unnecessary antibiotics in asymptomatic bacteriuria. Our three multicenter collaborative projects focus on 1) studies of the epidemiology of prophylactic antibiotic use in common surgeries and the impact of antibiotic use on AR, 2) in depth studies of the microbial etiology of mastectomy SSIs to understand the relationships between implants, biofilms and specific organisms and 3) studies of the relationship between AR recurrent UTIs and the human gut microbiome as a reservoir and predictor of AR UTIs and the impact of fecal microbiome transplant on AR UTIs and the gut microbiome.

*Overlap:* No overlap

#### *No change*

*Title:* Phylogenomic, Transcriptomic, Viromic, and Immuno-proteomic Determinants of Necrotizing

Enterocolitis *Time Commitment:* 1.08 Cal *Performance Period:* 09/06/17 – 05/31/22 *Level of Funding:*

*Supporting Agency:* NIH/NICHHD (Tarr; PI), GMS Jill Rogers

*Goals:* The major goals of this project are to determine the microbial and the host (infant) biology and ecology prior to the development of necrotizing enterocolitis.

*Aims:* Aim 1. Conduct a comprehensive genomic analysis of gut bacteria prior to NEC onset. Aim 2. Define the gut biology and host response prior to NEC onset.

*Overlap:* No overlap

#### *End date change*

*Title:* Systems Engineering of *Rhodococcus opacus* to Enable Production of Drop-in Fuels from Lignocellulose

*Time Commitment:* .96 Cal *Performance Period:* 09/15/17 – 09/14/21-NCE *Level of Funding:*

*Supporting Agency:* DOE, Office of Science Germantown Bldg., 1000 Independence Ave SW, DC, 20585.

Pablo Rabinowicz, 301-903-0379

*Goals:* The major goals of this project are to model, design, and engineer *R. opacus* mutants for the

production of biofuels and bioproducts from lignin.

*Aims:* This project has four main deliverables: (1) increased fundamental understanding of functional catabolic pathways and metabolic responses to diverse lignin-derived substrates and genetic modifications, (2) an enhanced genome-scale model of *R. opacus* which predicts internal metabolic flux confidence intervals from transcriptomic data, (3) synthetic expression control elements and multiplex genome editing tools for *R. opacus*, and (4) engineered *R. opacus* strains with improved abilities to convert lignin-derived substrates into drop-in biofuels: branched-chain fatty acid esters (BCFAEs).

*Overlap:* No overlap

### *No change*

*Title:* Occupational Exposure and Health Risk from Dairy Microbiome and Resistome to Dairy Farm Workers

*Time Commitment:* .96 Cal *Performance Period:* 09/01/18 – 08/31/22 *Level of Funding:* Supporting

*Agency:* CDC/NIOSH (PI/MPI: Dantas, Shukla)

*Goals:* The major goals of this project are to investigate the occupational exposure of dairy farm workers to antibiotic-resistant microorganisms (ARM) and infectious diseases from dairy cows.

*Aims:* Aim 1: Determine the shared and unique nasal and gut microbiota among dairy farm workers and dairy cows. Aim 2: Understand how nasal and fecal microbial communities, virulence factors, and resistomes, change temporally in dairy farm workers and dairy cows and examine the association between nasal/gut microbiome and seasonal incidence of influenza-like-illness (ILI), as well as gastrointestinal (GI) symptoms, in dairy workers. Aim 3: Quantitative analysis of acquisition, persistence, and transmission of antimicrobial resistant (AR) genes from dairy cows to farm workers. Aim 4: Develop a grounded understanding of dairy farm workers' attitudes, beliefs, and behaviors regarding their shared microbiome with dairy cows (the cows, their manure, and the environment)

*Overlap:* No overlap

### *No change*

*Title:* Tunable therapeutic modulation of the gut microbiome by engineered probiotics

*Time Commitment:* 1.38 Cal *Performance Period:* 08/10/18 – 06/30/23 *Level of Funding:* Supporting

*Agency:* NIH/NCCIH (Dantas; PI), GMS Mildred J. Qualls

*Goals:* The major goals of this project are to identify gene pathways and develop synthetic regulatory circuits that, when engineered into probiotics, robustly improve duration and tunability of therapeutic production by probiotics in the gut, and apply these technologies to the treatment of phenylketonuria.

*Aims:* 1) identify gene functions under selection in the gut, 2) design and optimize gut-relevant biosensors and kill switches, and 3) demonstrate the efficacy of in vivo delivery of a phenylketonuria (PKU) therapeutic by engineered probiotic strains.

*Overlap:* No overlap

### *No change*

*Title:* Novel Antibacterial Peptides Against Staphylococcus Aureus Via Nano-Culture Functional Metagenomics

*Time Commitment:* .06 Cal *Performance Period:* 05/02/18 – 04/31/21 *Subcontract Level of Funding:*

*Supporting Agency:* NIH/NIAID (Ramasubramanian; PI), GMS Sufiyan Saeed

*Goals:* The major goals of this project are to identify peptide molecules against growth, viability and biofilms of MRSA by screening genes from uncultured microbiomes using a novel ultra-high-throughput nanoculture platform.

*Aims:* (i) integrate functional metagenomics and nanoculture to develop a 'Library on a chip' consisting of a million unique clones on a single slide; (ii) interface the 'Library on a chip' with a 'Pathogen on a chip' nanoculture assay to prospect for peptides with antimicrobial activity including bactericidal and/or anti-biofilm effects against pathogenic *S. aureus*; (iii) annotate the sequences, and validate their proteolytic stability, toxicity, and activity against MRSA clinical isolates.

*Overlap:* No overlap

*No change (this award)*

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

*Time Commitment:* 1.80 Cal *Performance Period:* 09/30/18 – 09/29/21 *Level of Funding:* Supporting

*Agency:* DOD (Dantas; PI), USA Med Research ACQ Activity, 820 Chandler St., Fort Detrick, MD

21702-5014

*Goals:* The major goals of this project are 1) to enable focus of resources toward diarrhea-susceptible personnel and 2) to inform treatments which reduce the risk factors for diarrhea and antibiotic gene transmission.

*Aims:* Aim 1: Define the acquisition, persistence, and transmission of AR genes during travel to regions with high infectious disease burdens. We will test the hypothesis that infection by diarrheal pathogens and the use of antibiotics for prophylaxis or treatment of traveler's diarrhea causes shifts in gut microbiota composition, which results in increased MDRO colonization and persistence in affected travelers.

Aim 2: Understand host microbiota-pathogen dynamics during international travel to high infectious burden regions. We will test the hypothesis that certain keystone microbial communities in a traveler's microbiome are protective or permissive to infectious diarrhea, and that the presence of these keystone species in the microbiota preceding a diarrheal episode predicts disease outcome in international travelers.

Aim 3: Quantitatively analyze the long-term effect of antibiotic therapy on the gut microbiota and resistome in military personnel. We will test the hypothesis that antimicrobial treatment of infectious diarrhea will cause persistent perturbation in the gut microbiota characterized by shifts in phylogenetic composition and functional capacity, increased MDRO prevalence, and increased AR gene carriage in military personnel gut microbiomes. We further hypothesize that recovery of the gut microbiota following antibiotic treatment can be predicted based on the presence or abundance of specific biomarkers (e.g. particular organisms, pathways, or ARGs) before antibiotic exposure.

*Overlap:* This award

*No change*

*Title:* Genomic analysis of *C. difficile* transmission

*Time Commitment:* .36 Cal *Performance Period:* 03/01/20 – 02/28/21 *Level of Funding:* Supporting

*Agency:* Barnes Jewish Hospital Foundation (PI: Dubberke)

*Goals:* The major goal of this project is to characterize the potential transmission of *C. difficile* between patients and environmental surfaces.

*Aims:* We propose to leverage an observational study of *C. difficile* carriage, where strains were isolated from both patients and hospital surfaces. We will whole-genome sequence these *C. difficile* strains to determine the population structure of *C. difficile* in a hospital setting. Using quantitative genomics to determine strain relatedness, we will identify genetic elements of *C. difficile* associated with patient disease and transmissibility.

*Overlap:* No overlap

*New*

*Title:* Environmental hygiene strategies to decrease the burden of antibiotic-resistant organisms in Intensive Care Unit SINKs (ICU SINK)

*Time Commitment:* .96 Cal *Performance Period:* 07/01/20 – 06/30/25 *Level of Funding:* Supporting

*Agency:* AHRQ (PI/MPI: Kwon, Burnham, Dantas)

*Goals:* The major goals of this proposal are to 1) define ARO transmission between the hospital environment and patients, and 2) evaluate environmental hygiene strategies to reduce ARO burden in the ICU environment.

*Aims:* Aim 1: Longitudinally determine the presence and transmission of antibiotic resistant organisms (AROs) across the hospital environment and relate these strains to those causing blood stream infection in patients in a bone marrow transplant (BMT) intensive care unit (ICU). Aim 2: Implement and measure the impact of a bleach and hydrogen peroxide based environmental hygiene intervention on the concentration of AROs in the sinks present in the rooms of ICU patients.

*Overlap:* No overlap

*New*

*Title:* Multi-scale modeling and phylodynamics for healthcare associated infections

*Time Commitment:* .18 Cal *Performance Period:* 08/01/20 – 07/31/24 *Level of Funding:* Supporting  
*Agency:* NIH (NIDDK) (Lanzas, PI)

*Goals:* The major goals of this project are culturing and sequencing of 200 ESBL E. coli isolates, along with plasmid sequencing of 100 of these isolates obtained from the collection and cleaning of data from Barnes-Jewish Hospital.

*Aims:* Aim 1: Develop improved approaches for inferring routes of acquisition of HAI and optimizing HAI surveillance and control Aim 2: Phylodynamics to estimate fitness of antimicrobial resistance pathogens. Aim 3: Multi-scale models for multidrug-resistant organisms: extended-spectrum beta-lactamase (ESBL)-producing Enterobacteriaceae as case study.

PENDING

*New*

*Title:* The PRIME-biotic directive: Next-generation precision probiotics to live long and prosper

*Time Commitment:* 1.8 Cal *Performance Period:* 09/01/21 – 08/31/26 *Level of Funding:* Supporting  
*Agency:* NIH/OD (Dantas; PI)

*Goals:* The major goals of this project are to engineer precise and conditional therapeutic delivery systems in Escherichia coli Nissle 1917 (EcN) and Saccharomyces boulardii (Sb), enabling them to perform tumor-specific delivery of next-generation immune checkpoint inhibitors (ICIs) to treat colorectal cancer (CRC).

*Aims:* Aim 1: Define probiotic dynamics and TME characteristics within murine models of CRC; Aim 2: Expand the probiotic engineering toolbox to construct TME-specific ICI release mechanisms; Aim 3: Evaluate efficacy, tumor penetration, and toxicity of PRIME-biotics in a murine CRC model

*Overlap:* No overlap

*New*

*Title:* Probiotic-mediated delivery of recombinant PD-L1 antibody variants to the tumor microenvironment

*Time Commitment:* .30 Cal *Performance Period:* 01/01/21 – 12/31/22 *Level of Funding:* Supporting  
*Agency:* Siteman Cancer Center, Siteman Investment Program (Dantas; PI)

*Goals:* The major goals of this project are to engineer probiotic-mediated delivery of recombinant PD-L1 antibody variants to the tumor microenvironment

*Aims:* Aim 1: Engineer targeted yeast delivery systems of next-generation  $\alpha$ PD-L1 variants; ;Aim 2: Develop and characterize CRC in vivo models; Aim 3: In vivo testing of constitutively expressing ICI in mouse model of CRC

*Overlap:* No overlap

*New*

*Title:* Targeted delivery of anti-cancer therapies to the gastrointestinal tract achieved through a first-in-class dual probiotic system

*Time Commitment:* .84 Cal *Performance Period:* 01/01/21 – 12/31/22 *Level of Funding:* Supporting  
*Agency:* Siteman Cancer Center, Cancer Research Fund (Dantas; PI)

*Goals:* The major goals of this project are to engineer targeted delivery of anti-cancer therapies to the gastrointestinal tract achieved through a first-in-class dual probiotic system

*Aims:* Aim 1: Engineer Suite of Probiotic ICI Delivery Systems; Aim 2: Compare Functionality of Delivery Systems and Payloads In Vitro and Ex Vivo; Aim 3: Assess the Therapeutic Potential of Engineered Probiotic ICI Delivery Systems In Vivo

*Overlap:* No overlap

*New*

*Title:* Probiotic-mediated delivery of next-generation immune checkpoint inhibitors to the colorectal tumor microenvironment

*Time Commitment:* .60 Cal *Performance Period:* 01/01/21 – 12/31/21 *Level of Funding:*

*Supporting Agency:* Pardee Foundation (Dantas; PI)

*Goals:* The major goals of this project are to engineer probiotic-mediated delivery of next-generation immune checkpoint inhibitors to the colorectal tumor microenvironment

*Aims:* 1. Build a library of ICI variants and establish function after expression in yeast; 2. Initiate mouse model enriched for colonic tumors and assess expression of biomarkers directly relevant to probiotic ICI delivery system; 3. Engineer protease-dependent ICI release system in Sb that delivers ICI directly to TME

*Overlap:* No overlap

### *New*

*Title:* Klebsiella pneumoniae Virulence Regulation by RfaH and NusG Homologs

*Time Commitment:* .12 Cal *Performance Period:* 07/01/21 – 06/30/23 *Level of Funding:* Supporting  
*Agency:* NIH/NIAID (Rosen; PI)

*Goals:* These studies will explore the role of RfaH and similar homologs in regulating genes important for Klebsiella pneumoniae pathogenesis. In addition to defining and characterizing the regulome of RfaH and similar NusG homologs a lux assay will be used to screen for inhibitors of RfaH function with the goal of developing anti-virulence therapeutics.

*Aims:* Aim 1. Define and characterize the regulome of RfaH and other NusG homologs in Kp.; Aim 2. Identify small-molecule inhibitors of RfaH function and their effects on Kp virulence.

*Overlap:* No overlap

### *No change*

*Title:* Investigating gut microbiome composition and functions during stages of Alzheimer's disease

*Time Commitment:* .18 Cal *Performance Period:* 10/01/20 – 09/30/21 *Level of Funding:* Supporting  
*Agency:* Infectious Diseases Society of America (IDSA) (Dantas; PI)

*Goals:* To test the hypothesis that cognitively normal but amyloid positive individuals at risk of Alzheimer disease have variant gut microbiome composition and functional activity

*Aims:* AIM 1: Characterize gut taxonomic composition and diversity in adults at different stages of AD. AIM 2: Test for association of specific microbial taxa with AD status and AD biomarker levels. AIM 3: Characterize GM transcriptional activity and identify functional pathways associated with AD severity.

*Overlap:* No overlap

### *No change*

*Title:* Antecedent biomarkers for Alzheimer disease: The adult children study (Project 3)

*Time Commitment:* .96 Cal *Performance Period:* 06/01/21 – 05/31/26 *Level of Funding:* Supporting  
*Agency:* NIH/NIA (Morris; PI)

*Goals:* ACS Project 3 will perform longitudinal analyses of gut microbiome composition, microbial function, and intestinal permeability and inflammation and compare these measures with Alzheimer Disease biomarkers in ACS participants.

*Aims:* Aim 1: Characterize gut bacterial content and gut dysfunction (↑ permeability and inflammation) in adults at different stages of AD. Aim 2: Identify longitudinal changes in gut bacterial content and gut dysfunction (↑ permeability and inflammation) in CN-healthy and CN-preclinical AD individuals, and people with symptomatic AD. Aim 3: Associate gut bacterial content and gut dysfunction (↑ permeability and inflammation) with AD biomarkers obtained from other Projects and Cores of the PPG.

*Overlap:* No overlap

### *No change*

*Title:* Washington University & BJC Epicenter to Prevent Healthcare Associated Infections & Antibiotic Resistance

*Time Commitment:* .84 Cal *Performance Period:* 09/30/20 – 09/29/25 *Level of Funding:* Supporting  
*Agency:* CDC (Fraser, PI)

*Goals:* The Washington University (WU) / BJC Prevention Epicenter (PE) works collaboratively with CDC & other PEs to perform research & translate discoveries into novel strategies to prevent healthcare-associated

infections (HAI) & antimicrobial resistance (AR). The WU/BJC PE is widely recognized for HAI & AR research & leadership. WU has outstanding research infrastructure. BJC is an integrated healthcare delivery system with 15 hospitals, long-term care facilities, & outpatient practices. BJC includes two academic hospitals, Barnes-Jewish & St. Louis Children's Hospitals & 13 suburban & rural hospitals. We organized a research team with expertise in ID, epidemiology, informatics, microbiology, critical care, microbiome, & metabolism to study HAI & AR in diverse settings. We developed a high impact 5-yr proposal with novel strategies to: Evaluate & predict inappropriate antimicrobial use & AR regionally & nationally; Reduce surgical site infections; Test novel initiatives to improve outpatient antibiotic prescribing; Identify metagenomic & metabolite differences between hospital & community associated *C. difficile*; Reduce AR organism reservoirs in hospitals.

*Aims:* Aim 1A: Evaluate the use, effectiveness, and safety of antimicrobials in community acquired pneumonia (CAP) using national administrative data. Aim 1B: Develop aggregate antimicrobial resistance measures in sepsis caused by gram-negative bacilli (GNB) using BJC electronic health record (EHR) data. Aim 2: The impact of an existing Anesthesia Control Tower (ACT) intervention to improve intraoperative care on infectious outcomes. Aim 3A: Stewardship initiative to limit post-discharge prophylactic antibiotic use after mastectomy. Aim 3B: Improve antimicrobial stewardship practices (ASP) in non-traditional outpatient populations, specifically elderly patients with UTIs in assisted living facilities. Aim 4: Identify fecal microbiome and metabolite markers of community associated (CA) *Clostridioides difficile* colonization and infection (CDI). Aim 5: Define the prevalence of antibiotic resistant organisms (ARO) in the hospital ICU environment, their relationship to ARO in clinical culture isolates and measure the impact of an environmental hygiene intervention on ARO concentration in ICU sink drains, the surrounding environment and bioaerosols.

*Overlap:* No overlap

### *No change*

*Title:* Structural, mechanistic, and evolutionary characterization of tetracycline destructases

*Time Commitment:* 1.8 Cal    *Performance Period:* 02/01/21 – 01/31/26    *Level of Funding:* Supporting

*Agency:* NIH (NIAID) (PI/MPI: Dantas, Wencewicz)

*Goals:* The major goals of this project are to characterize the structure, mechanism and evolutionary profiles of the tetracycline destructases, a novel family of tetracycline inactivating enzymes.

*Aims:* Aim 1: Elucidate the mechanism of tetracycline inactivation by the tetracycline destructases. Aim 2: Understand the evolution of tetracycline destructases at genetic and population levels. Aim 3: Develop inhibitors and diagnostic agents for tetracycline destructases.

*Overlap:* No overlap

### COMPLETED

*Title:* Double blinded, randomized controlled Trial of Oral vancomycin versus placebo in hospitalized patients with diarrhea and stool toXin NEGative but nucleic acid amplification test positive for toxigenic *Clostridium difficile* (TOX NEG trial)

*Time Commitment:* .60 Cal    *Performance Period:* 09/30/17 – 09/29/19    *Level of Funding:* Supporting

*Agency:* CDC, Office of Acquisition Services, 2920 Brandywine Rd, RM 3000, Atlanta, GA 30341, Elizabeth Wilkins, 404-718-4729 (Dubberke; PI)

*Goals:* The primary purpose of this work is to conduct a double-blinded randomized controlled trial of CDI treatment (oral vancomycin 125mg, four times/day for ten days) versus matching placebo among patients whose stools are NAAT-positive / toxin-negative. To assess the risk-benefit of CDI treatment, we will determine the impact of oral vancomycin on the microbiome, antimicrobial resistance, *C. difficile* and MDRO colonization and environmental contamination, duration of diarrhea, and CDI-related adverse outcomes (e.g. death) versus placebo.

*Aims:* Aim 1) Determine if there are differences in microbiome disruption and acquisition / persistence of *C. difficile* and other MDRO carriage in stool among hospitalized patients whose stools are NAAT-positive / toxin-negative who are randomized to a 10-day course of oral vancomycin compared to placebo. Aim 2) Determine if there are differences in *C. difficile* and other MDRO environmental contamination between

treatment groups. Aim 3) Determine whether there is a difference in CDI-related outcomes among study participants who receive oral vancomycin compared to placebo.

*Overlap:* No overlap

*Title:* Quantifying the Impacts of Antibiotic Prophylaxis on Antimicrobial Resistance (AMR) in Vulnerable Pediatric Populations in Africa.

*Time Commitment:* .12 Cal      *Performance Period:* 11/01/16 – 04/30/18      *Level of Funding:*

*Supporting Agency:* Bill & Melinda Gates Foundation, Steven Buchsbaum, Deputy Director, PO Box 23350, Seattle, WA 98102

*Goals:* The major goal of this project is to quantify AMR gene enrichment due to short-term and long-term prophylactic antibiotics in children with severe acute malnutrition or exposure to HIV.

*Aims:* We will quantify antimicrobial resistance gene enrichment due to short-term and long-term prophylactic antibiotics in African children with severe acute malnutrition or exposure to HIV.

*Overlap:* No overlap

*Title:* Systems Biology of *Rhodococcus opacus* to Enable Production of Fuels and Chemicals from Lignocellulose

*Time Commitment:* .60 Cal      *Performance Period:* 09/01/14 – 08/31/18 (NCE)      *Level of Funding:*

*Supporting Agency:* Department of Energy, DE-FOA-0001060, Program Manager Joseph R. Graber, DoE, Office of Acquisition and Assistance, 9800 South Cass Avenue, Argonne, IL 60439

*Goals:* The major goal of this project is to understand the systems biology of *Rhodococcus opacus* to enable production of fuels and chemicals from lignocellulose.

*Aims:* Aim 1. Understand native genetic networks in *R. opacus* for metabolizing phenolic compounds. Aim 2. Interrogate phenolic tolerance mechanisms in *R. opacus* via high-throughput gain-of-function and loss-of-function screening. Aim 3. Identify preferred phenolic substrates in *R. opacus* from thermochemically depolymerized lignin and the governing metabolic regulation by combining subtractive metabolic profiling and transcriptomics.

*Overlap:* No overlap

*Title:* Quantitative Modeling of Antibiotic Resistance Gene Transmission Between Human and Environmental Microbiota

*Time Commitment:* .60 Cal      *Performance Period:* 10/01/14 – 09/30/18      *Level of Funding:* *Supporting*

*Agency:* Edward Mallinckrodt Jr. Foundation, Rebecca Blankenship, Director, 114 Forest Grove Drive, Glen Carbon, IL 62034

*Goals:* The major goal of this project is to quantitatively model antibiotic resistance gene transmission between human and environmental microbiota.

*Aims:* To advance the understanding of natural resistance exchange networks by identifying the antibiotic resistance genes (ARGs) most commonly shared between human and environment, examining the genetic context of these shared genes, and quantifying how habitat and phylogeny impact their distributions

*Overlap:* No overlap

*Title:* Multidrug-Resistant Pathogen Surveillance in Pakistani and U.S. Hospitals

*Time Commitment:* .36 Cal      *Performance Period:* 09/15/15 – 08/31/18      *Level of Funding:* *Supporting*

*Agency:* USAID/National Academy of Sciences, Garland Anderson, NAS Sub-Grant Manager, 500 Fifth Street, NW, Washington, DC 20001

*Goals:* The major goal of this project is to compare multidrug resistant pathogens in Pakistani and U.S. hospitals.

*Aims:* Aim 1: Compare the pathogens recovered and antibiotic resistance genes identified from surface samples from Pakistani and U.S. hospitals. Aim 2: Identify organisms in patient samples from both U.S. and Pakistani hospitals. Aim 3: Build capacity in Pakistan to perform antibiotic resistance tracking.

*Overlap:* No overlap

*Title:* Establishment and Dynamics of Antibiotic Resistance Reservoirs in Developing Intestinal Microbiota

*Time Commitment:* .60 Cal *Performance Period:* 12/28/12 – 11/30/17 (NCE) *Level of Funding:*

*Supporting Agency:* NIH (NIGMS), GMS Erica West

*Goals:* The major goal of this project is to investigate adaptive dynamics and genetic exchange of resilience functions in pediatric gut microbiota.

*Aims:* Aim 1: Characterize development of the intestinal resistome in healthy twin pairs during the critical, and developmentally dynamic, first two years of life. Aim 2: Define effects of early antibiotic therapy on the intestinal resistome of very-low birth weight (VLBW) infants.

*Overlap:* No overlap

*Title:* Enduring Effects of Early Life Antibiotic Therapy on Microbiota Development and Antibiotic Resistance Gene Carriage in the Preterm Infant Gut

*Time Commitment:* 1.2 Cal *Performance Period:* 09/15/16 – 09/14/17 *Level of Funding:* *Supporting Agency:* CDC, 200-2016-91955, Kristopher Lemaster, Office of Acquisition Services, 2920 Brandywine Road, Atlanta, GA 30341-5539

*Goals:* The objectives of this project are 1) to quantitatively analyze long-term effects of early life antibiotic therapy on the preterm infant gut microbiota and 2) to assess the reproducibility of antibiotic disruptions in gnotobiotic mouse model of early life antibiotic therapy.

*Aims:* Aim 1: Quantitatively analyze the long-term effects of antibiotic therapy on the preterm infant gut microbiome. Aim 2: Assess ability to reproduce antibiotic disruptions of infant gut microbiota in gnotobiotic mouse model of early life antibiotic therapy.

*Overlap:* No overlap

*Title:* Prospective Study Characterizing Fecal Microbiome Disruptions during and after receipt of Antimicrobials

*Time Commitment:* .84 Cal *Performance Period:* 09/30/16 – 09/29/17 *Level of Funding:* *Supporting Agency:* CDC (Kwon, PI), 200-2016-91962, Kristopher Lemaster, Office of Acquisition Services, 2920 Brandywine Road, Atlanta, GA 30341-5539

*Goals:* The goal of this study is to characterize microbiome disruptions (from baseline) observed during and after usual courses of antimicrobials.

*Aims:* Aim 1: Determine the effects of antimicrobials used to treat community acquired pneumonia (CAP) on the fecal microbiota architecture and resistome (i.e. compendium of antibiotic resistance genes within the microbiota) in healthy adults. Aim 2: Determine the time to restoration of the fecal microbiome after exposure to antimicrobials used to treat CAP. Aim 3: Quantitatively predict microbiota disruption based on 1) the degree of fecal microbiota disruption, 2) duration of microbiota disruption, and 3) resistome expansion.

*Overlap:* No overlap

*Title:* The Fecal Microbiome and Resistome of Patients with Multidrug-Resistant UTIs. This project is funded through the Pilot Projects in Translational and Clinical Sciences Program of the Washington University Institute of Clinical and Translational Sciences (ICTS).

*Time Commitment:* 0 Cal *Performance Period:* 06/01/16 – 05/31/17 *Level of Funding:* *Supporting Agency:* CTSA#905 (Kwon – Pilot Project PI) Washington University ICTS (Evanoff, PI)

*Goals:* The gastrointestinal (GI) tract is a key source of bacteria that contaminate the periurethral area and cause urinary tract infections (UTI). The proposed research will create a biospecimen repository to define the microbial communities that reside in the GI tract of patients with MDRO UTIs. These findings may ultimately result in the development of tools to identify patients at risk for MDRO UTIs, and subsequent prevention interventions.

*Aims:* Aim 1: Define the trajectory of the fecal microbiome in patients with MDRO UTI. Aim 2: Identify microbial markers that are protective from, or permissive to persistent MDRO colonization and recurrent MDRO UTIs.

*Overlap:* No overlap

*Title:* Metagenomic Engineering of Probiotic Bacteria to Improve Intestinal Colonization Dynamics and Relative Fitness

*Time Commitment:* 3.0 Cal *Performance Period:* 09/30/12 – 06/30/17 *Level of Funding:* Supporting  
*Agency:* NIH/NIDDK (Director's New Innovator Award), GMS Florence Danshes

*Goals:* The major goal of this project is to develop a novel platform technology for engineering fitness-enhancing properties into probiotic bacteria under direct in vivo selection in a mammalian host.

*Aims:* We will functionally prospect complete human microbiota and defined gut microbiota culture collections for genes and operons facilitating integration (collectively referred to as integration-enhancing fragments or IEFs) of a set of candidate probiotics into stable microbiota in mammalian hosts, investigate the mechanisms of the identified genes, and compete and combinatorically optimize the best IEFs to engineer enhanced integration phenotypes.

*Overlap:* No overlap

*Title:* Rapid and Accurate Sequencing-Based Diagnostics for Urinary Tract Infections

*Time Commitment:* 0 Cal *Performance Period:* 06/01/15 – 05/31/16 *Level of Funding:* Supporting  
*Agency:* Barnes Jewish Hospital/Washington University Institute of Clinical and Translational Science, Donald Buckner, Grants and Funds Administration Manager, 1001 Highlands Plaza Drive West, Suite 140, St. Louis, MO 63110

*Goals:* The major goal of this project is to develop a rapid and accurate sequencing-based diagnostic for UTIs

*Aims:* Aim 1: Characterize and compare the diversity of the UTI microbial community via shotgun DNA sequencing between three different sampling methodologies: single isolates vs. culturable bacteria vs. culturable and non-culturable bacteria. Aim 2: Characterize the resistome of UTI samples to inform prudent antibiotic therapy in the future.

*Overlap:* No overlap

*Title:* Synthetic engineering of enhanced fitness and adhesion properties in probiotics for the treatment of inflammatory bowel disease (IBD)

*Time Commitment:* .75 Cal *Performance Period:* 10/01/12 – 09/30/15 *Level of Funding:* Supporting  
*Agency:* Kenneth Rainin Foundation (Breakthrough Award), Pat Curcio, VP & Program Officer, 155 Grand Avenue, Suite 1000, Oakland, CA, 94612

*Goals:* The major goal of this project is to engineer improved probiotics for the treatment of IBD.

*Aims:* We will engineer enhanced fitness and adhesion properties in probiotics for the treatment of inflammatory bowel disease.

*Overlap:* No overlap

*Title:* Washington University and BJC Epi-Center for Prevention of Healthcare Associated Infections, Antimicrobial Resistance and Adverse Events

*Time Commitment:* .60 Cal *Performance Period:* 09/01/14 – 08/31/15 *Level of Funding:* Supporting  
*Agency:* CDC (Fraser, PI) Sheila Edwards, Procurement and Grants Office, Koger Center, Stanford Building, 2920 Brandywine Road, Atlanta, GA 30341

*Goals:* The major goal of this project is to evaluate the impact of fecal transplantation for *C. difficile* treatment on resistant organism carriage and the resistome.

*Aims:* Aim 1: Define the fecal microbiota architecture, resistome, and MDRO carriage of people who have been exposed to multiple courses of antimicrobials. Aim 2: Define the short and long term impacts of allogeneic FMT on the fecal microbiota architecture, resistome, and MDRO carriage. Aim 3: Determine the component(s) of the fecal microbiota associated with both protection from and susceptibility to colonization with MDRO and expansion of the resistome.

*Overlap:* No overlap

*Title:* Effects of early antibiotic therapy on the antibiotic resistome in the intestinal microbiota of very-low birth weight infants in the neonatal ICU

*Time Commitment:* 1.8 Cal *Performance Period:* 06/01/12 – 05/31/15 *Level of Funding:*

*Supporting Agency:* March of Dimes, Debbi Bailey-Graff, Director, Grants Administration, 1275 Mamaroneck Avenue, White Plains, NY 10605

*Goals:* The major goal of this project is to characterize the effects of early antibiotic therapy on the microbiota of premature infants.

*Aims:* We aim to test the hypotheses that systemic antibiotic therapy administered to preterm infants will (1) decrease the phylogenetic diversity of the gastrointestinal microbiota, (2) apply selective pressure to generate a population-wide increase in the number and diversity of antibiotic resistance genes (ARGs) in this community, and (3) these changes will persist long after the cessation of antibiotic therapy.

*Overlap:* No overlap

*Title:* Functional characterization of antibiotic resistance reservoirs in developing pediatric microbiomes

*Time Commitment:* 2.4 Cal *Performance Period:* 02/01/11 – 01/31/15 *Level of Funding:* Supporting

*Agency:* Children's Discovery Institute Interdisciplinary Research Initiative, Angela Corless, Executive Administrator, Campus Box 8208, 660 South Euclid Avenue, St. Louis, MO 63110

*Goals:* The major goals of this project are to develop and optimize novel, high-throughput methods for investigating microbial functions in the pediatric microbiota, leveraging advances in functional metagenomics and next-generation sequencing.

*Aims:* Year One: Aims 1 and 2: At the end of year one, we hope to have completed development of our proposed massively parallel sequencing approach for characterizing antibiotic resistomes, and demonstrated its feasibility to characterize the antibiotic resistomes of a pilot cohort of 22 pediatric fecal samples. Year Two: Aim 2: At the end of year two, we hope to have characterized the resistomes from the first six months of samples of infant twin pairs and their mothers, and establish the appropriate temporal sampling resolution required for following resistome dynamics in this study. Aim 3: At the end of year two, we hope to have defined the effects of early antibiotic therapy on the prevalence and diversity of the antibiotic resistome in the intestinal microbiota of very-low birth weight infants in the NICU. Year Three Aim 2: At the end of year three, we hope to understand the environmental and genetic factors that influence the establishment and dynamics of the antibiotic resistomes in intestinal microbiota of healthy newborns and infants. Aim 3: At the end of year three, we hope to understand whether constrained starting bacterial diversity combined with sustained broad-spectrum antibiotic therapy will enrich for very specific antibiotic resistance determinants in the intestinal microbiota of VLBW infants surviving NEC, compared to disease-free and antibiotic-free counterparts.

*Overlap:* No overlap

*Title:* Antibiotic supplementation for severe malnourishment in children

*Time Commitment:* .12 Cal *Performance Period:* 12/31/13 – 12/30/14 *Level of Funding:* Supporting

*Agency:* Medecins Sans Frontiers – Epicentre, Emmanuel Baron, 8 rue Saint Saban, 75011 Paris, France

*Goals:* The goal of this project is to study the impact of antibiotic supplementation for severe malnourishment in children.

*Aims:* We aim to elucidate the impact on gut microbiota composition of routine antibiotic use in the outpatient treatment of severely malnourished children without complications.

*Overlap:* No overlap

*Title:* Microbial Ecosystem Services: Identifying global solutions from genes to communities

*Time Commitment:* 0 Cal *Performance Period:* 05/01/12 – 09/1/14 *Level of Funding:* Supporting

*Agency:* National Academies Keck Futures Initiative Ecosystem Services Grant, Phyllis Rhoades, Assistant Director, Center Administration, PO Box 775, Cambridge, MD 21613

*Goals:* The major goal of this project is to hold a workshop that will provide an opportunity for scientist to discuss solutions to socio-environmental problems which may involve the direct or indirect modulation of genes and communities, or some degree of engineering of microbial processes.

*Aims:* We aim to hold a scientific workshop which will result in (1) at least one collaborative, synthesis paper that summarizes key knowns and unknowns in microbial ecosystem services, (2) graduate student training, and,

(3) teaching and communication resources in the form of an open-source database of key literature and publications for teaching microbial ecosystem services.

*Overlap:* No overlap

Overlap

Should any of the pending applications be awarded, the level of effort will be adjusted, if needed, so it does not exceed 100%.

OTHER SUPPORT SIMONS, MARK

ACTIVE

*No change*

*Title:* Application of personalized phage cocktails for treatment of *Staphylococcus aureus* induced osteomyelitis/bone infection in a rat model blast-related extremity injury: route of administration, dose response, and tissue distribution

*Time Commitment:* 10% *Performance Period:* 10/2017-09/2021 *Level of Funding:*

*Supporting Agency:* Defense Health Programs

*Supporting Agency Contracting/Grants Officer Name and Address:* Silver Spring, MD

*Role:* Co-Principal Investigator

*Goals:* Development of bacteriophage library as a life-saving treatment of antibiotic resistant infections

**There is no overlap with the proposed project.**

*No change – this award*

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

*Time Commitment:* 10% *Performance Period:* 10/2017-09/2021 *Level of Funding:*

*Supporting Agency:* Congressionally Directed Medical Research Programs

*Supporting Agency Contracting/Grants Officer Name and Address:* Silver Spring, MD

*Goals:* Investigate gut microbiota-pathogen interactions and subsequent carriage of MDROs during international travel to high infectious disease burden regions

*No change*

*Title:* Characterization of T-cell responses following burn and blast-related extremity injury

*Time Commitment:* 5% *Performance Period:* 10/2017-09/2021 *Level of Funding:*

*Supporting Agency:* Defense Health Programs

*Supporting Agency Contracting/Grants Officer Name and Address:* Silver Spring, MD

*Role:* Co-Investigator

*Goals:* Determine mechanism of immune suppression following blast and burn injuries

**There is no overlap with the proposed project.**

*No change*

*Title:* Development of machine learning algorithms in animal models for prediction of wound outcomes in humans *Time*

*Commitment:* 10% *Performance Period:* 10/2017-03/2021 *Level of Funding:*

*Supporting Agency:* Defense Health Programs

*Supporting Agency Contracting/Grants Officer Name and Address:* Silver Spring, MD

*Role:* Principal Investigator

*Goals:* Development of predictive computational models to predict wound healing outcomes

**There is no overlap with the proposed project.**

*No change*

*Title:* Fixed and Modularized Bacteriophage Cocktails for Treatment of Combat-Trauma Associated Infections Caused by Multidrug-resistant ESKAPE Bacterial Pathogens

*Time Commitment:* 20% *Performance Period:* 10/2019-09/2023 *Level of Funding:* *Supporting Agency:*

Congressionally Directed Medical Research Programs

*Supporting Agency Contracting/Grants Officer Name and Address:* Silver Spring, MD

*Role:* Principal Investigator

*Goals:* Development and preclinical evaluation of bacteriophage products to treat combat trauma associated infections cause by multidrug resistant bacterial pathogens. IND-enabling studies towards early human clinical trials.

**There is no overlap with the proposed project.**

*No change*

*Title:* Preclinical Evaluation of novel antibacterial approaches against ESKAPE pathogens in animal models of infection

*Time Commitment:* 10% *Performance Period:* 10/2019-09/2022 *Level of Funding:*

*Supporting Agency:* Defense Health Programs

*Supporting Agency Contracting/Grants Officer Name and Address:* Silver Spring, MD

*Role:* Associate Investigator

*Goals:* Support for preclinical evaluation of antimicrobial products, developed by government and external partners, to be used in the field and during medical transport which will reduce microbial pathogens and protect the tissue viability in warfighters with poly-traumatic wounds infections.

**There is no overlap with the proposed project.**

*No change*

*Title:* Probing the host immune system response during phage therapy to determine parameters for optimal efficacy

*Time Commitment:* 10%    *Performance Period:* 10/2019-09/2021    *Level of Funding:*

*Supporting Agency:* Defense Health Programs

*Supporting Agency Contracting/Grants Officer Name and Address:* Silver Spring, MD

*Role:* Principal Investigator

*Goals:* Development of predictive computational models to predict wound healing outcomes

**There is no overlap with the proposed project.**

*No change*

*Title:* Implementation of Gene Therapy to Accelerate Combat Wound Healing

*Time Commitment:* 5%    *Performance Period:* 10/2019-09/2021    *Level of Funding:*

*Supporting Agency:* Defense Health Programs

*Supporting Agency Contracting/Grants Officer Name and Address:* Silver Spring, MD

*Role:* Associate Investigator

*Goals:* Development of predictive computational models to predict wound healing outcomes

**There is no overlap with the proposed project.**

*No change*

*Title:* Integration of antimicrobials into FDA-approved Keracis fish skin for advanced wound care

*Time Commitment:* 20%    *Performance Period:* 10/2019-09/2021    *Level of Funding:*

*Supporting Agency:* Defense Health Programs

*Supporting Agency Contracting/Grants Officer Name and Address:* Silver Spring, MD

*Role:* Principal Investigator

*Goals:* Development of predictive computational models to predict wound healing outcomes

**There is no overlap with the proposed project.**

PENDING

None

COMPLETED

*New*

*Title:* Combat Wound Infection Multidrug-Resistant (MDR) and other Virulent Organisms Initiative – Trauma Infectious Diseases Outcome Study (TIDOS)

*Time Commitment:* 10%    *Performance Period:* 10/2017-09/2020    *Level of Funding:*

*Supporting Agency:* Defense Health Programs

*Supporting Agency Contracting/Grants Officer Name and Address:* Silver Spring, MD

*Role:* Associate Investigator

*Goals:* Evaluate role of Enterococcus species in combat-associated traumatic wounds

**There is no overlap with the proposed project.**

*Title:* Development of murine infection wound model to assess therapeutics against gram negative biofilms

*Time Commitment:* 5%    *Performance Period:* 10/2017-09/2018    *Level of Funding:*

*Supporting Agency:* Defense Health Programs

*Supporting Agency Contracting/Grants Officer Name and Address:* Silver Spring, MD

*Role:* Associate Investigator

*Goals:* Development of model to advance treatment to target biofilms of antibiotic resistant infections

*Title:* Investigation of novel antimicrobials for prevention and treatment of infections with water-borne pathogens  
*Time Commitment:* 10% *Performance Period:* 10/2017-09/2019 *Level of Funding:*  
*Supporting Agency:* Defense Health Programs  
*Supporting Agency Contracting/Grants Officer Name and Address:* Silver Spring, MD  
*Role:* Co-Investigator  
*Goals:* Development antimicrobial wound dressings for use in battlefield settings

*Title:* Promising New Therapeutic Approaches to Wound Infection: Improving Efficacy through Incorporation into Tunable Nanofiber Wound Dressings  
*Time Commitment:* 10% *Performance Period:* 10/2017-09/2018 *Level of Funding:*  
*Supporting Agency:* Defense Health Programs  
*Supporting Agency Contracting/Grants Officer Name and Address:* Silver Spring, MD  
*Role:* Co-Principal Investigator  
*Goals:* Development antimicrobial wound dressings for use in battlefield settings

*Title:* Assessment of human microbiome associated with enteric diseases and wound infections among deployed military personnel  
*Time Commitment:* 10% *Performance Period:* 10/2016-09/2017 *Level of Funding:*  
*Supporting Agency:* Navy Advanced Medical Development Program  
*Supporting Agency Contracting/Grants Officer Name and Address:* Silver Spring, MD  
*Role:* Principal Investigator  
*Goals:* Integrate microbiome analysis into existing studies to evaluate the microflora changes during wound and enteric infections

*Title:* Epidemiology and characterization of bacterial pathogens associated with wound infections in Peru  
*Time Commitment:* 5% *Performance Period:* 10/2014-09/2016 *Level of Funding:*  
*Supporting Agency:* Armed Forces Health Surveillance Center/Global Emerging Infections Surveillance (GEIS)  
*Supporting Agency Contracting/Grants Officer Name and Address:* Silver Spring, MD  
*Role:* Principal Investigator  
*Goals:* Active surveillance of the epidemiology and etiology of wound infections among Peruvian hospitals including antibiotic resistance patterns

*Title:* Epidemiology of nosocomial infections and characterization of molecular resistance factors from Latin American hospitals  
*Time Commitment:* 5% *Performance Period:* 10/2014-09/2016 *Level of Funding:*  
*Supporting Agency:* Armed Forces Health Surveillance Center/Global Emerging Infections Surveillance (GEIS)  
*Supporting Agency Contracting/Grants Officer Name and Address:* Silver Spring, MD  
*Role:* Co-Investigator  
*Goals:* Active surveillance of the epidemiology and etiology of wound infections among Peruvian hospitals including antibiotic resistance patterns

*Title:* Epidemiology and etiology of acute gastroenteritis in US military deployed to Latin America  
*Time Commitment:* 10% *Performance Period:* 10/2014-09/2016 *Level of Funding:*  
*Supporting Agency:* Armed Forces Health Surveillance Center/Global Emerging Infections Surveillance (GEIS)  
*Supporting Agency Contracting/Grants Officer Name and Address:* Silver Spring, MD  
*Role:* Principal Investigator  
*Goals:* Passive surveillance of diarrhea among U.S. military troops to investigate prevalence by etiology

*Title:* Epidemiology of Deployment-Associated Diarrhea at US Joint Task Force Bravo, Honduras: establishment of baseline data for future vaccine and intervention trials  
*Time Commitment:* 10% *Performance Period:* 10/2014-09/2016 *Level of Funding:*  
*Supporting Agency:* Military Infectious Diseases Research Program (MIDRP)  
*Supporting Agency Contracting/Grants Officer Name and Address:* Frederick, MD  
*Role:* Principal Investigator  
*Goals:* Active surveillance of the incidence, etiology, operational impact, and long-term effect on health-related quality of life among U.S. military personnel deployed to Honduras

*Time Commitment:* 10%    *Performance Period:* 10/2014-09/2016    *Level of Funding:*  
Supporting Agency: Armed Forces Health Surveillance Center/Global Emerging Infections Surveillance (GEIS)  
*Supporting Agency Contracting/Grants Officer Name and Address:* Silver Spring, MD  
*Role:* Co-Investigator  
*Goals:* Passive surveillance of diarrhea among U.S. Embassy personnel and travelers

*Title:* Development of an electronic surveillance system in Latin American countries for detection of the causes of enteric diseases and patterns of antimicrobial resistance

*Time Commitment:* 5%    *Performance Period:* 10/2013-09/2014    *Level of Funding:*  
Supporting Agency: Armed Forces Health Surveillance Center/Global Emerging Infections Surveillance (GEIS)  
*Supporting Agency Contracting/Grants Officer Name and Address:* Silver Spring, MD  
*Role:* Co-Investigator  
*Goals:* Establishment of electronic database for passive surveillance of enteric pathogens and antibiotic resistance patterns among foreign military laboratories

*Title:* Viral Etiology and Viral Load in Persons with Non-Bacterial Non-Parasitic Acute Gastrointestinal Illness and Asymptomatic Controls In Peru

*Time Commitment:* 5%    *Performance Period:* 10/2014-09/2015    *Level of Funding:*  
Supporting Agency: Armed Forces Health Surveillance Center/Global Emerging Infections Surveillance (GEIS)  
*Supporting Agency Contracting/Grants Officer Name and Address:* Silver Spring, MD  
*Role:* Associate Investigator  
*Goals:* Analysis of archived stool samples for undetected viral etiologies using multiplex PCR methods

*Title:* Implementation and evaluation of the field performance of the FilmArray biosurveillance system in Cuzco and Puerto Maldonado, Peru

*Time Commitment:* 5%    *Performance Period:* 10/2013-09/2014    *Level of Funding:*  
Supporting Agency: Congressional Medical Development Funds  
*Supporting Agency Contracting/Grants Officer Name and Address:* Washington, DC  
*Role:* Principal Investigator  
*Goals:* Evaluation of the Biofire FilmArray for respiratory and diarrhea pathogen detection in jungle and high-altitude environments