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TITLE: Bacteriophage Therapies to Prevent and Treat Diarrhea in Military Personnel

PRINCIPAL INVESTIGATOR: Anika Kinkhabwala, Ph.D.

RECIPIENT: EpiBiome, Inc., South San Francisco, CA 94080

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TYPE OF REPORT: Final

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

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REPORT DOCUMENTATION PAGE

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Anika Kinkhabwala

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E-Mail: anika@epibiome.com

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EpiBiome, Inc.
29528 Union City Blvd
Union City, CA 94587-1245

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14. ABSTRACT

Our goal was to help improve military productivity by developing bacteriophage-based treatments that can be used instead of antibiotics. Bacteriophages, or "phages", are small viruses that infect bacteria and are harmless to humans. EpiBiome's automated phage discovery pipeline was to be leveraged to find 100 phages against enterotoxigenic E. coli, each of which would then be characterized in detail to identify the best phages for a therapeutic phage cocktail.

15. SUBJECT TERMS

Bacteriophage, ETEC, antibiotic resistance, diarrhea

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1. **INTRODUCTION:** Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.

Our goal was to help improve military productivity by developing bacteriophage-based treatments that can be used instead of antibiotics. Bacteriophages, or "phages", are small viruses that infect bacteria and are harmless to humans. EpiBiome's automated phage discovery pipeline was to be leveraged to find 100 phages against enterotoxigenic E. coli, each of which would then be characterized in detail to identify the best phages for a therapeutic phage cocktail.

2. **KEYWORDS:** Provide a brief list of keywords (limit to 20 words).

Bacteriophage, ETEC, antibiotic resistance, diarrhea

3. **ACCOMPLISHMENTS:** The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction.

What were the major goals of the project?
List the major goals of the project as stated in the approved SOW. If the application listed milestones/target dates for important activities or phases of the project, identify these dates and show actual completion dates or the percentage of completion.

Aim 1:
Automated phage discovery pipeline to isolate > 100 phages - 100% complete
Characterize relevant aspects of phage infection to identify candidate cocktail phages - 57% complete

Aim 2:
Sequence phage genomes - 41% complete
Compare phage genome sequences - 41% complete

Aim 3:
Isolate phage resistant hosts - 40% complete
Sequence genomes of resistant and susceptible hosts - 10% complete
Compare genomes to identify resistance mutations - 10% complete

Aim 4:
Collate experimental and genomic data to select cocktail phages - 0% complete
Test cocktails *in vitro* - 0% complete

What was accomplished under these goals?

For this reporting period describe: 1) major activities; 2) specific objectives; 3) significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative); and/or 4) other achievements. Include a discussion of stated goals not met. Description shall include pertinent data and graphs in sufficient detail to explain any significant results achieved. A succinct description of the methodology used shall be provided. As the project progresses to completion, the emphasis in reporting in this section should shift from reporting activities to reporting accomplishments.

Aim 1.

The primary goal of Aim 1 was to use our automated phage discovery platform to isolate 100 ETEC phages and characterize their host range. The first step was to optimize our automated phage discovery pipeline for discovering phages against ETEC bacterial strains. This required integrating multiple custom automated systems and writing code for both system operation as well as data management and automated data analysis.

Environmental samples are the sources of all phages we used for this project, and thus, efficient collection, storage, and organization of these samples was essential for the success of this project. Sampled environments include, but are not limited to, wastewater, hospital waste, feces, and soil. During this project we finalized a sample processing protocol, which includes documenting all samples in a Laboratory Information Management System (LIMS). Each incoming sample was barcoded, filtered to remove bacteria and other debris, and then stored at a temperature appropriate for long-term viability. Samples were stored in tubes from which aliquots could be easily loaded into the phage discovery pipeline. Our sample collection currently has over 1200 samples.

One of the main components of this automated phage discovery pipeline is culturing bacteria and tracking their growth using robots for automated incubation, shaking, and optical density readings. Productive infection by phages results in bacterial lysis; thus, measuring the optical densities of bacterial cultures is an efficient way to identify environmental samples that contain phages capable of lysing a host of interest. As shown in Figure 1, the automated pipeline produces a growth curve for each well of a 96-well plate and also automatically analyzes the data to pick out the most promising samples for further isolation and amplification.

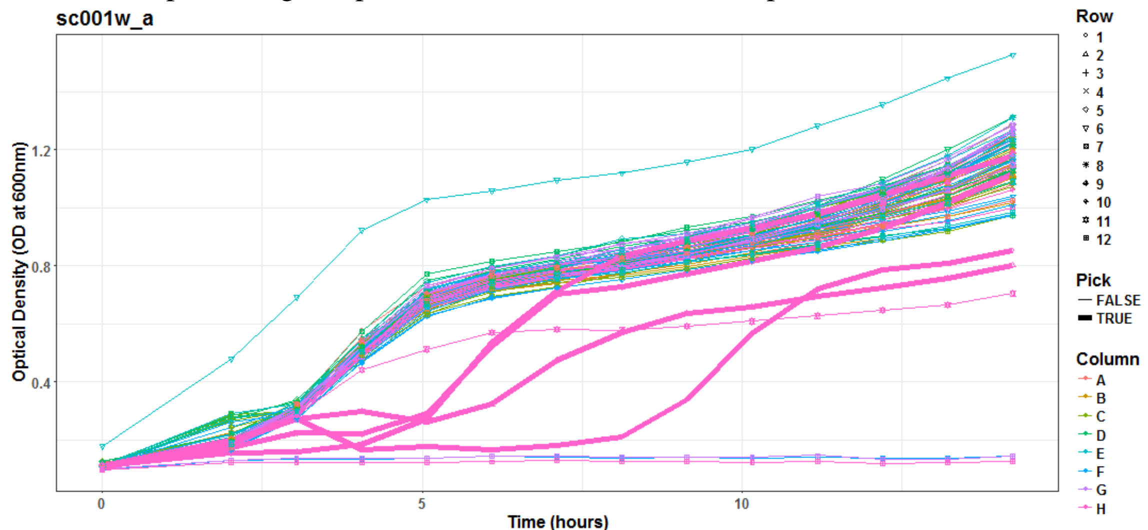

Figure 1. Automated optical density readings as a function of time for each well in a 96-well plate. The same ETEC bacterial strain was added to each well along with an aliquot of an environmental sample to determine whether that environmental sample contained one or more phages able to kill the host (detected as a decreased optical density, and indicated with thick lines, “TRUE”). The system is capable of processing multiple 96-well plates simultaneously.

The pipeline processes these growth curve data to produce “area under the curve” calculations that can then be used to call “hits”, i.e., whether an environmental sample contains a phage that kills the ETEC host strain. This calculation is performed for every well in the 96-well plate as follows: 1) The mean “blank” control is calculated for every time capture point from those wells containing only buffer (F12, G12, and H12). This mean control value represents a background response which is subtracted from every growth curve. 2) Areas under the growth curve are calculated by integrating the curve from 2 to 6 hours. 3) The mean “growth” control is calculated from well containing bacteria but no phage (A12, B12, C12, D12, and E12). 4) Final scores are calculated by dividing each test well value by the value for mean “growth” control. Thus, final scores represent the fraction of expected growth, and any environmental sample producing a final score less than 0.7 is labeled a “hit” (see Figure 2 for example).

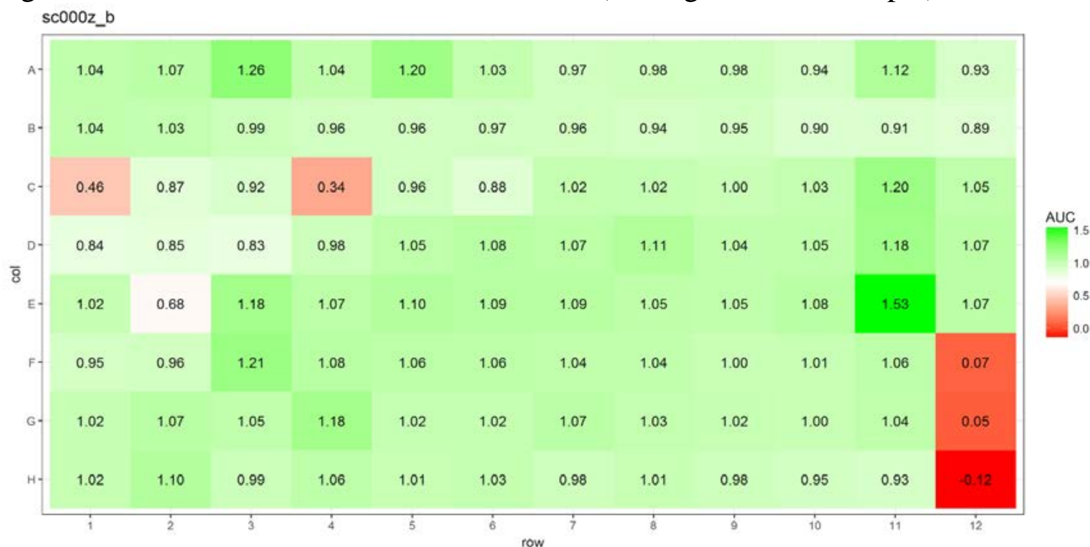

Figure 2. Area under the curve (AUC) calculations for each well in a 96-well plate, calculated from growth curve data such as those shown in Figure 1. AUC controls for differences in initial culture density and enables efficient identification of wells, i.e., environmental samples, that warrant further investigation. In this case, wells F12, G12, and H12 represent controls where there should be low or zero AUC. Low AUC values (in red) indicate limited bacteria growth and therefore are potential hits (wells C1, E2, and C4)

The screening process that identifies hits for one 96-well plate takes about 10 hours to complete. Test plates are created on a liquid handling robot and automatically transferred to a fully automated incubator system. While one plate incubates, additional plates of the same samples can be created against additional bacterial strains and loaded into the incubator. After an initial incubation period, plates are automatically transferred from the incubator to a spectrophotometer for optical density readings and automatically transferred back to the incubator once readings are complete. The system is capable of handling up to 20 plates simultaneously for a total daily throughput of 1,760 independent screening tests.

Over the course of the past year, 107 ETEC phage hits were identified, thus completing the first part of Aim 1. In order to perform host range testing and sequencing, however, required titers of at least 10^7 PFU/mL, which meant that many of the phages were not fully analyzed.

Phage Hits	Isolated Phages	Host Range Testing	Completed Genomes
107	98	57	41

Table 1: Summary of ETEC Phage Discovery efforts to date.

The best phages for therapeutic uses have a large host-range. There are several methods to measure host range, including but not limited to optical density readings, spot clearing and efficiency of plating (EOP). EOP was selected as the most useful for therapy, since its requirement of efficient plaquing for calling a phage hit is likely the most stringent requirement of all the techniques. In EOP, a phage is plaque tittered on every bacterial strain of interest in order to measure what percentage of phages create plaques for that bacterial strain. Table 2 shows the results of this testing, which, unfortunately, demonstrated that there is a limited host range for all the ETEC phages discovered.

Phage discovery was optimized in several ways to attempt to find broader host-range phages. In one set of experiments, instead of enriching on several bacterial strains at once, each environmental sample was enriched against just one bacterial strain per night sequentially. It was hoped that this protocol would bias towards phages that could successfully amplify every night and thus on every strain, but the phages discovered in this protocol did not achieve larger host ranges. After these experiments, it was noted that almost all the phages isolated to date were isolated on one ETEC bacterial strain, b00dr. If this strain was distinct from the rest of the panel, it might explain the host-range issue, so a new effort began to isolate phages against the other strains. It was more difficult to isolate phages against these strains and, in the end, their host range was not any higher (typically 1-2 strains). In the end, the inability to find ETEC phages with medium to large host ranges prevented the formation of a cocktail for Aim 4, which was a suprising results based on EpiBiome's previous experience with E. coli cocktails. The best explanation for this result is that the ETEC panel was formed from bacteria from international sources, while our environmental panel is from strictly domestic sources. Perhaps phages that target these strains are only found internationally, but it was not possible to source wastewater samples from these regions in time for completion of this project.

		Host strain											
	PHAGE ID	b00dr	b00du	b00dv	b00dw	b00dx	b00dy	b00dz	b00e0	b00e1	b00e2	b00e3	b00cq
bdr a8-1	p0053												
bdr a8-2	p0054												
bdr a9-1	p0056												
bdr a9-2	p0057												
bdr a9-3	p0058												
bdr bk-1	p005h												
bdr bh-1	p005d												
bdr br-1	p005e												
bdr bg-1	p005j												
bdy bp-1	p005y												
bdr bf-1	p005k												
bdr bf-2	p005m												
bdr bf-4	p005q												
bdr bp-3	p005t												
bdr VC1-1	p005z												
bdr VC1-2	p0060												
bdr VC1-3	p0061												
bdr VC2-1	p0062												
bdx WT1-1	p0064												
bdr WT1-1	bdr WT1-1												
bdr WT1-2	bdr WT1-2												
bdr WT2-1	bdr WT2-1												
bdx WT1-2	bdx WT1-2												
bdy WT1-1	bdy WT1-1												
p66	p0066												
p67	p0067												
p68	p0068												
p69	p0069												
p6a	p006a												
p6b	p006b												
p6c	p006c												
p6d	p006d												
p6e	p006e												
p6g	p006g												
p6h	p006h												
p6j	p006j												
p6k	p006k												

	PFU/mL
	no infection
	1e1 - 1e2
	1e2 - 1e3
	1e3 - 1e4
	1e4 - 1e5
	1e5 - 1e6
	1e6 - 1e7
	1e7 - 1e8
	1e8 - 1e9
	1e9 - 1e10
	1e10 - 1e11
	1e11 - 1e12
	>1e12

		Host strain											
	PHAGE ID	b00dr	b00du	b00dv	b00dw	b00dx	b00dy	b00dz	b00e0	b00e1	b00e2	b00e3	b00cq
p6m	p006m												
p4k	p004k												
p4m	p004m												
p4p	p004p												
p6y	p006y												
p6z	p006z												
po	p000o												
p70	p0070												
p71	p0071												
p72	p0072												
pn	p000n												
bdz E11	p007f												
bdz G2	p007g												
bdz G7	p007h												
bdz G8	p007j												
bdz H2	AAABA_b_H2												
bdz H4	AAABA_b_H4												
bdz H5	p007k												
bdz H6	p007m												
bdz H7	p007p												
bdz H11	p007q												

Table 2: Host range of 57 ETEC phages using EOP.

Aim 2.

Aim 2 activities focused on sequencing the genomes of all the ETEC phages. Phage sequencing begins with phage gDNA extraction, which was performed on phage lysates using the Phage DNA Isolation Kit (Norgen Biotek Corp, Thorold, ON, Canada) following the manufacturer's protocol. Any remaining host DNA was degraded by adding 10 microliters (20 U) of DNase I from the RNase-free DNase I kit (Norgen Biotek Corp, Thorold, ON, Canada) prior to Proteinase K treatment. Extracts were prepared for sequencing on the Illumina MiSeq platform (Illumina, San Diego) using the Nextera XT Library Preparation Kit (Illumina, San Diego) according to the manufacturer's protocol (Part # 15031942, revision D). The magnetic bead normalization step was replaced with a manual normalization step, based on library concentration and average size as measured by the Qubit 3.0 fluorometer and Qubit dsDNA High Sensitivity Kit (Thermo Fisher Scientific, Waltham, MA) and the Fragment Analyzer (AATI, Ankeny, Iowa), respectively. Paired-end sequencing was performed using the MiSeq Reagent v3 (600 cycle) kit (Illumina, San Diego). Every phage was assembled with EpiBiome's phage informatics pipeline.

A phage with an assembled genome can be compared to every other discovered phage using the MASH genomic similarity algorithm[1]. Figure 3 shows this metric for every phage pair, which clearly identifies the most dissimilar phages to use in a cocktail. This figure easily demonstrates that a wide variety of phages were discovered in Aim 1 and genomic diversity was not the reason for the poor host range.

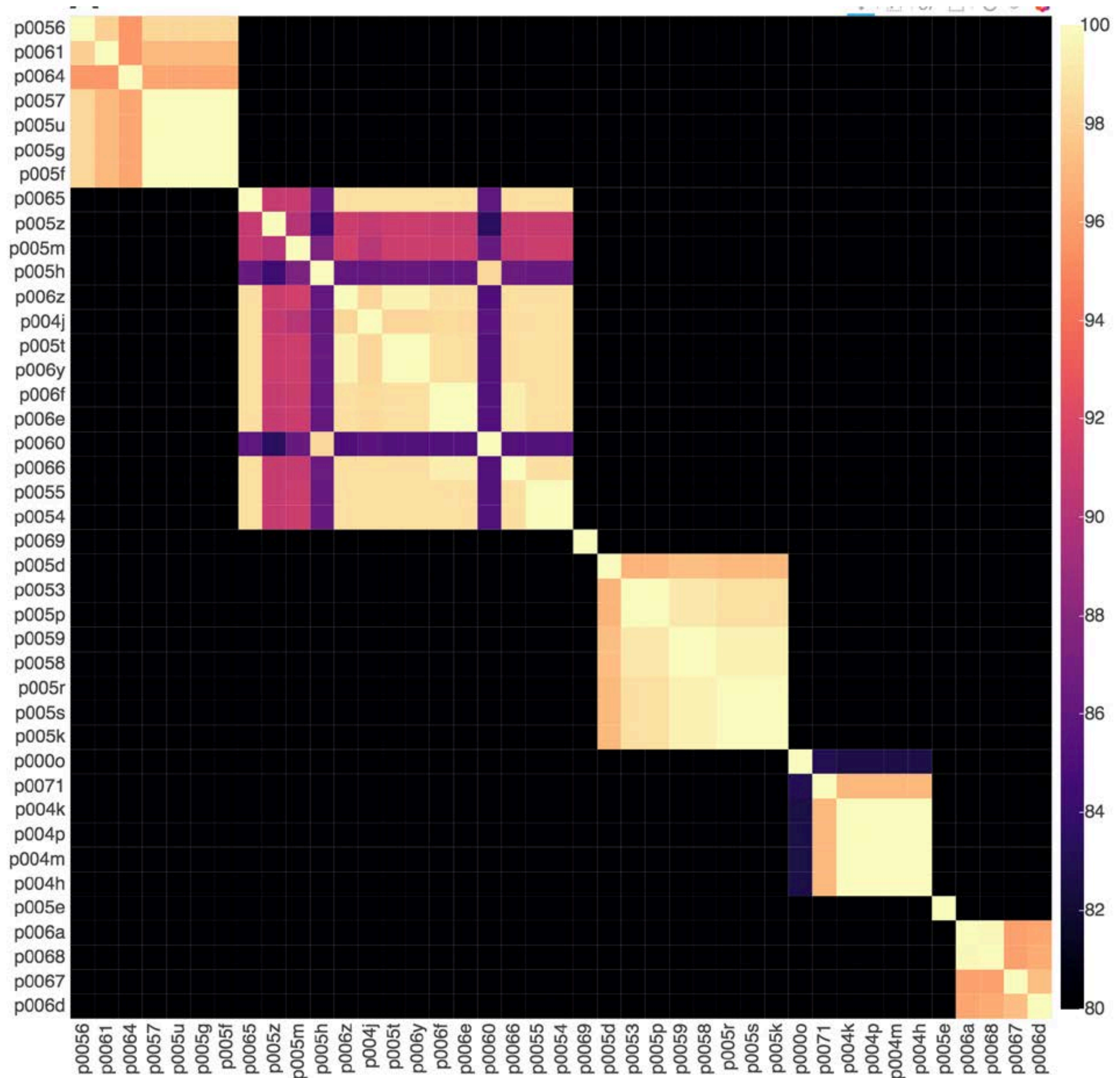

Figure 3: Genomic similarity, as calculated using the MASH algorithm[1], for every sequenced phage.

References:

1. Ondov, Brian D., et al. "Mash: fast genome and metagenome distance estimation using MinHash." *Genome biology* 17.1 (2016): 132.

Aim 3

Aim 3 preliminary work included sequencing and analyzing the genomes of bacteriophage resistant ETEC mutants to identify the mutation(s) that enabled each mutant to resist phage infection (Table 2). This information can be used to understand how the phage infects the bacterial host, and phages that infect using different mechanisms are ideal for treatment cocktails. The genome analysis results suggest that lipopolysaccharide (LPS) on the

bacterial cell surface has been altered, which likely enabled the bacteria to “hide” from the phage. The goal of this laborious experiment was the validate the high-throughput TnSeq method, which would be developed in this project in order to understand the phage-host interactions for all discovered phages.

Table 2. Bacteriophage insensitive mutants isolated and sequenced as of 08-25-17.

Phage	**Host**	**Mutant number**	**Putative cause of phage resistance: *gene name* (mutation), predicted effect on gene sequence**	**Function of gene product**
p004h	b00dr	1	*rfaC* (M1R), no protein	LPS biosynthesis
		2	*rfaC* (Δ392bp), truncated protein	LPS biosynthesis
		3	*rfaE* (Δ22bp), truncated protein	LPS biosynthesis
		4	to be determined	
		5	to be determined	
		6	to be determined	
		7	to be determined	
		8	to be determined	
		9	to be determined	
p0057	b00dr	1	*rfaL* (S57*), truncated protein	LPS biosynthesis
		2	*rfaL* (L375*), truncated protein	LPS biosynthesis
		3	*rfbC* (W98*), truncated protein	LPS biosynthesis
		4	*queD* (Δ1bp), truncated protein	Nucleoside biosynthesis
		5	to be determined	
		6	to be determined	
		7	to be determined	
		8	to be determined	
		9	to be determined	

Tn-Seq is a high-throughput approach designed to identify all non-essential genes potentially involved in the development of phage resistance in a bacterial cell. In Tn-seq, a single gene of a single bacterial cell is disrupted by randomly inserting a transposon into it. Random insertions into many cells creates a library of mutants, such that every cell in the library contains a single transposon that interrupts the genome in a different way (depicted as differently colored cells in Figure 3). The addition of phage pressure selects for mutants that can resist phage infection. Transposon-guided PCR and sequencing of the surviving mutants (that resisted phage infection) then identifies the transposon-interrupted gene for the mutants that evaded phage infection, thereby identifying genes involved in phage resistance across many mutants in a single experiment. The key to this technique is creating a diverse mutant library, which means a mutant library with at least 10,000 mutants. TnSeq libraries were created against two ETEC strains, but were not validated in time for this project.

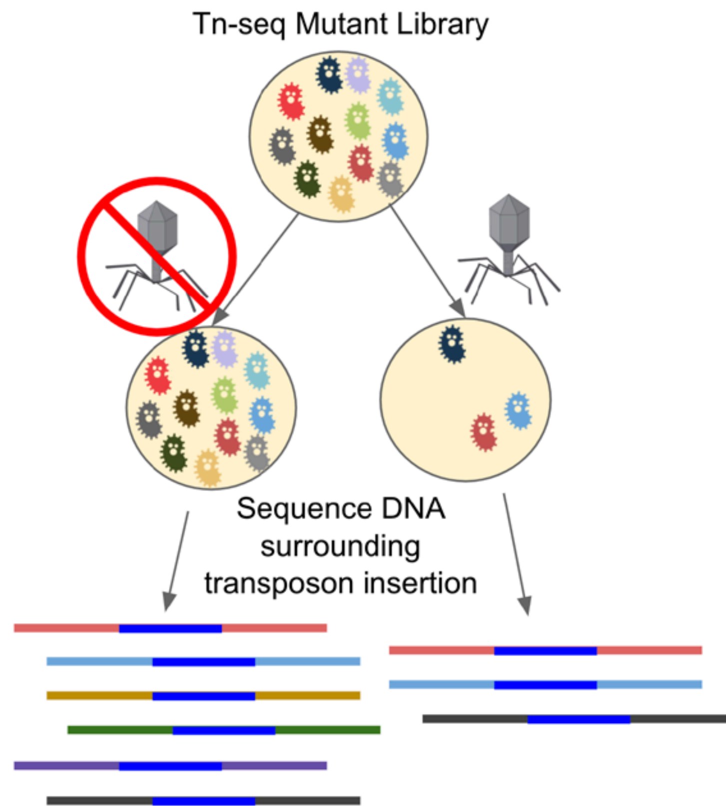

Figure 3: Tn-seq: a library of mutants is exposed to phage (or not, for a no-phage control) under defined conditions. By sequencing the DNA surrounding the transposon insertion of survivors, those genes whose mutations confer resistance to the phage are readily identified.

Aim 4

Since medium to large host-range phages could not be discovered in Aim 1, it was not possible to formulate a useful ETEC cocktail from this project. It should be noted that these phages do have higher host-ranges against other E. coli indications.

What opportunities for training and professional development has the project provided?

If the project was not intended to provide training and professional development opportunities or there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe opportunities for training and professional development provided to anyone who worked on the project or anyone who was involved in the activities supported by the project. "Training" activities are those in which individuals with advanced professional skills and experience assist others in attaining greater proficiency. Training activities may include, for example, courses or one-on-one work with a mentor. "Professional development" activities result in increased knowledge or skill in one's area of expertise and may include workshops, conferences, seminars, study groups, and individual study. Include participation in conferences, workshops, and seminars not listed under major activities.

Anika Kinkhabwala attended two sequencing conferences hosted by Oxford Nanopore Technologies.

How were the results disseminated to communities of interest?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the results were disseminated to communities of interest. Include any outreach activities that were undertaken to reach members of communities who are not usually aware of these project activities, for the purpose of enhancing public understanding and increasing interest in learning and careers in science, technology, and the humanities.

Nothing to Report.

What do you plan to do during the next reporting period to accomplish the goals?
If this is the final report, state "Nothing to Report."

Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.

Nothing to Report.

4. **IMPACT:** Describe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:

What was the impact on the development of the principal discipline(s) of the project?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how findings, results, techniques that were developed or extended, or other products from the project made an impact or are likely to make an impact on the base of knowledge, theory, and research in the principal disciplinary field(s) of the project. Summarize using language that an intelligent lay audience can understand (Scientific American style).

Nothing to Report.

What was the impact on other disciplines?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the findings, results, or techniques that were developed or improved, or other products from the project made an impact or are likely to make an impact on other disciplines.

Nothing to Report.

What was the impact on technology transfer?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe ways in which the project made an impact, or is likely to make an impact, on commercial technology or public use, including:
- *transfer of results to entities in government or industry;*
- *instances where the research has led to the initiation of a start-up company; or*
- *adoption of new practices.*

EpiBiome used the knowledge gained from this project to streamline our E. coli phage discovery process and the phages isolated in this project, while not ideal for an ETEC cocktail, have been found to have high host ranges against other E. coli indications.

What was the impact on society beyond science and technology?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how results from the project made an impact, or are likely to make an impact, beyond the bounds of science, engineering, and the academic world on areas such as:
- *improving public knowledge, attitudes, skills, and abilities;*
- *changing behavior, practices, decision making, policies (including regulatory policies), or social actions; or*
- *improving social, economic, civic, or environmental conditions.*

Nothing to Report.

5. **CHANGES/PROBLEMS:** The PD/PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, "Nothing to Report," if applicable:

Changes in approach and reasons for change

Describe any changes in approach during the reporting period and reasons for these changes. Remember that significant changes in objectives and scope require prior approval of the agency.

Nothing to Report.

Actual or anticipated problems or delays and actions or plans to resolve them

Describe problems or delays encountered during the reporting period and actions or plans to resolve them.

There were two significant challenges encountered in this project. Firstly, and most importantly, all phages discovered against the ETEC bacterial strains in our library had extremely narrow host-ranges. We attempted to tweak our phage discovery protocol in a few ways to optimize for larger host range phages. First, we attempted to enrich on multiple bacterial strains sequentially, hoping that only phages that could amplify on all of the bacterial strains would win out, but phages discovered in this manner also had a narrow host range. Next, we noted that most of our phages were isolated on one bacterial strain (b00dr), which was especially easy to find phages against, so we changed our efforts and attempted to isolate phages against bacterial strains that had not phage hits. These phages, while being able to hit the one bacterial strain they were discovered against, rarely hit more than one other bacterial strain, so also had a narrow host range. Overall, we were not successful in creating a cocktail with significant host range over our ETEC panel, which was a surprising result since it has historically been easy for EpiBiome to create E. coli cocktails for other indications. The most likely cause is that the ETEC strains assembled for this panel are international in origin and we did not have access to foreign wastewater samples for phage discovery.

Secondly, in Aim 3, the approach was updated to utilize TnSeq, a high-throughput method that would allow all phages to be tested. Significant progress was made to create the required transposon library, but we were not able to complete the method development in time to demonstrate the technique and test it on the many phages discovered in the course of this project.

Changes that had a significant impact on expenditures

Describe changes during the reporting period that may have had a significant impact on expenditures, for example, delays in hiring staff or favorable developments that enable meeting objectives at less cost than anticipated.

Nothing to Report.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Describe significant deviations, unexpected outcomes, or changes in approved protocols for the use or care of human subjects, vertebrate animals, biohazards, and/or select agents during the reporting period. If required, were these changes approved by the applicable institution committee (or equivalent) and reported to the agency? Also specify the applicable Institutional Review Board/Institutional Animal Care and Use Committee approval dates.

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:** List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report."

- **Publications, conference papers, and presentations**
  Report only the major publication(s) resulting from the work under this award.

Journal publications. *List peer-reviewed articles or papers appearing in scientific, technical, or professional journals. Identify for each publication: Author(s); title; journal; volume: year; page numbers; status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

Nothing to Report.

Books or other non-periodical, one-time publications. *Report any book, monograph, dissertation, abstract, or the like published as or in a separate publication, rather than a periodical or series. Include any significant publication in the proceedings of a one-time conference or in the report of a one-time study, commission, or the like. Identify for each one-time publication: author(s); title; editor; title of collection, if applicable; bibliographic information; year; type of publication (e.g., book, thesis or dissertation); status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

Nothing to Report.

Other publications, conference papers and presentations. *Identify any other publications, conference papers and/or presentations not reported above. Specify the status of the publication as noted above. List presentations made during the last year (international, national, local societies, military meetings, etc.). Use an asterisk (*) if presentation produced a manuscript.*

Anika Kinkhabwala presented at Oxford Nanopore Sequencing Day, March 12, 2018, "Understanding Phage-Bacterial Host Interactions for Smarter Therapeutics"

Anika Kinkhabwala presented at Oxford Nanopore Community Meeting, December 2017, "Understanding Phage-Bacterial Host Interactions for Smarter Therapeutics"

- **Website(s) or other Internet site(s)**
  List the URL for any Internet site(s) that disseminates the results of the research activities. A short description of each site should be provided. It is not necessary to include the publications already specified above in this section.

Nothing to Report.

- **Technologies or techniques**
  Identify technologies or techniques that resulted from the research activities. Describe the technologies or techniques were shared.

EpiBiome's phage discovery pipeline was optimized for discovering E. coli phages and could be used in general to discovery E. coli phages against any indication, not just ETEC. This technology was not publicly shared, however.

- **Inventions, patent applications, and/or licenses**
  Identify inventions, patent applications with date, and/or licenses that have resulted from the research. Submission of this information as part of an interim research performance progress report is not a substitute for any other invention reporting required under the terms and conditions of an award.

Nothing to Report.

- **Other Products**
  Identify any other reportable outcomes that were developed under this project. Reportable outcomes are defined as a research result that is or relates to a product, scientific advance, or research tool that makes a meaningful contribution toward the understanding, prevention, diagnosis, prognosis, treatment and /or rehabilitation of a disease, injury or condition, or to improve the quality of life. Examples include:
  - *data or databases;*
  - *physical collections;*
  - *audio or video products;*
  - *software;*
  - *models;*
  - *educational aids or curricula;*
  - *instruments or equipment;*

- *research material (e.g., Germplasm; cell lines, DNA probes, animal models);*
- *clinical interventions;*
- *new business creation; and*
- *other.*

Phages discovered in this project were found to have large host ranges against other E. coli indications , such as UTI's and adherent invasive E. coli, and are being used to create cocktails for these indications.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort). If information is unchanged from a previous submission, provide the name only and indicate "no change".

Example:

Name: *Mary Smith*
Project Role: *Graduate Student*
Researcher Identifier (e.g. ORCID ID): *1234567*
Nearest person month worked: *5*

Contribution to Project: *Ms. Smith has performed work in the area of combined error-control and constrained coding.*
Funding Support: *The Ford Foundation (Complete only if the funding support is provided from other than this award.)*

Name: Anika Kinkhabwala, Ph.D.
Project Role: PI
Resarcher ID: 0000-0001-9839-1350
Nearest person month worked: 12
Contribution to project: Coordinated all activities, including phage discovery, host range testing and sequencing of phages. Also, performed all bioinformatics on phages and bacteria related to the project.

Name: William Van Trump
Project Role: Director of Automation
Nearest person month worked: 1
Contribution to project: Automated phage discovery pipeline for discovering ETEC phages.

Name: Ryan Honaker
Project Role: Manager of Molecular and Microbiology
Nearest person month worked: 0.5
Contribution to project: Coordinated phage discovery of ETEC phages

Name: Zachary Hobbs
Project Role: RA
Nearest person month worked: 1
Contribution to project: Mr. Hobbs operated the automated phage discovery pipeline to find new ETEC phages.

Name: Maciej Zaczek
Project Role: RA
Nearest person month worked: 1
Contribution to project: Mr. Zaczek performed experiments to characterize phages, grew resistant bacterial mutants and contributed to developing the phage discovery pipeline.

Name: Rebecca Lu
Project Role: Manager of Molecular and Microbiology
Nearest person month worked: 3
Contribution to project: Sequenced phages and coordinated phage banking.

Name: Eyra Dordi
Project Role: RA
Nearest person month worked: 2
Contribution to project: Titering of ETEC phages and measurements of OD host range.

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

If the active support has changed for the PD/PI(s) or senior/key personnel, then describe what the change has been. Changes may occur, for example, if a previously active grant has closed and/or if a previously pending grant is now active. Annotate this information so it is clear what has changed from the previous submission. Submission of other support information is not necessary for pending changes or for changes in the level of effort for active support reported previously. The awarding agency may require prior written approval if a change in active other support significantly impacts the effort on the project that is the subject of the project report.

Nothing to Report.

What other organizations were involved as partners?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe partner organizations – academic institutions, other nonprofits, industrial or commercial firms, state or local governments, schools or school systems, or other organizations (foreign or domestic) – that were involved with the project. Partner organizations may have provided financial or in-kind support, supplied facilities or equipment, collaborated in the research, exchanged personnel, or otherwise contributed.

Provide the following information for each partnership:
Organization Name:
Location of Organization: (if foreign location list country)
Partner's contribution to the project (identify one or more)

- *Financial support;*
- *In-kind support (e.g., partner makes software, computers, equipment, etc., available to project staff);*
- *Facilities (e.g., project staff use the partner's facilities for project activities);*
- *Collaboration (e.g., partner's staff work with project staff on the project);*
- *Personnel exchanges (e.g., project staff and/or partner's staff use each other's facilities, work at each other's site); and*
- *Other.*

Organization Name: Ohio State University, Prof. Matt Sullivan
Location of Organization:
Partner's contribution to the project: Facilities, Collaboration

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: N/A

QUAD CHARTS: N/A

9. APPENDICES: N/A