Environment and Natural Resources Trust Fund
2018 Request for Proposals (RFP)

Project Title: **ENRTF ID: 088-B**

Maximizing Water Safety against Antibiotic Resistance

Category: B. Water Resources

Total Project Budget: $ 350,000

Proposed Project Time Period for the Funding Requested: 2 years, July 2018 to June 2020

Summary:

The proposed research will establish comprehensive experimental and informational framework for surveillance and monitoring of the effects of antibiotic use on the spread of antibiotic resistance.

Name: Arkady Khodursky

Sponsoring Organization: U of MN

Address: Biotechnology Institute 1479 Gortner Ave. 140 Gortner Labs
St. Paul MN 55108

Telephone Number: (612) 625-3799

Email khodu001@umn.edu

Web Address

Location

Region: Statewide

County Name: Statewide

City / Township:

Alternate Text for Visual:

Antibiotics and antibiotic resistant bacteria (ARB) can be released into Minnesota water environment through wastewater treatment plants and agricultural water run-offs. In this proposal, we will develop new DNA assays to detect various ARB, use these assays in high-throughput format to analyze spread of the ARB in various water environments, identify the source of the spread, and create information resource to assist local managers and regulators.

_____ Funding Priorities	_____ Multiple Benefits	_____ Outcomes	_____ Knowledge Base
_____ Extent of Impact	_____ Innovation	_____ Scientific/Tech Basis	_____ Urgency
_____ Capacity Readiness	_____ Leverage	_____ TOTAL	_____%

PROJECT TITLE: Maximizing water safety against antibiotic resistance

I. PROJECT STATEMENT

The goal of this project is to establish comprehensive experimental and informational framework for surveillance and monitoring of the effects of antibiotic use on the spread of antibiotic resistance. The project will (1) establish new, quantitative indicators for antibiotic effects on the spread of resistance, (2) identify the source of the spread, and (3) contribute to the development of guidelines that are aimed at controlling antibiotic footprint and its consequences on public health.

While antibiotic treatments save millions of lives each year around the world, nearly a century of antibiotic use and misuse has resulted in the evolution of resistance of bacterial pathogens to most antibiotics currently in use. Domestic wastewater can contain antibiotics and antibiotic resistant bacteria (ARB). However, current wastewater treatment plants are not designed to remove antibiotics and ARB. As a result, these contaminants are released into the environment, which is of great public health concern. In addition, agriculture can also contribute to the spread of antibiotics and ARB in the environment.

Despite a well-established need for antibiotic stewardship and emergence of the corresponding guideline protocols, reliable data on antibiotic prescription and consumption are difficult to obtain and the available usage information is highly variable. We propose that the molecular patterns of antibiotic resistance can serve as fingerprints of antibiotic usage and that such patterns may serve as a means for: i) assessing existing threats posed by ARB, ii) detecting the emergence and spread of previously uncommon or completely novel types of resistance, iii) integrating analysis of the spread of antibiotic resistance in the state, iv) sharing information about antibiotic resistance among the interested parties.

In this project, we will comprehensively detect multiple antibiotic resistance genes (ARGs) in various water samples, by using high-throughput gene detection technology. In addition, we will target the resistance genes associated with fluoroquinolone (FQ) antibiotics. FQ's belong to a family of synthetic broad-spectrum antibiotics, which are widely used in clinical and agricultural practices and, due to their potency, represent the antibacterial defense of last resort. Moreover, the use of these antibiotics has been recently associated with the development of resistance to other antibacterials and with persistent communal and hospital infections.

II. PROJECT ACTIVITIES AND OUTCOMES

Activity 1: *Sample collection and isolation of FQ-resistant bacteria* **Budget: $110,000**

We will obtain samples from various water samples from lakes and rivers in Minnesota. Sites include several wastewater treatment facilities, several locations along St. Louis and Minnesota Rivers, which runs through agricultural areas, recreational beaches facing to the Duluth-Superior Harbor and Lake Superior, as well as from multiple water reuse systems around the state. Overall, 300 samples will be collected, including 10 samples during the course of one year from each site, to analyze temporal profiling of the ARGs.
Bacteria resistant to FQ antibiotics will be screened and isolated. In addition, DNA will be extracted from the water samples, and used for the gene detection assays in Activity 3.

Outcome	Completion Date
1. Sample collection and DNA extractions	September 30, 2019
2. Isolation of FQ-resistant bacteria	November 30, 2019

Activity 2: *Assay development* **Budget: $45,000**

We will develop new quantitative PCR (qPCR) assays to detect genes associated with FQ resistance for *Escherichia coli* and other major waterborne pathogens. Currently, there is no comprehensive tool available to analyze FQ resistance, mostly due to limited number of gene information associated with FQ resistance. To obtain genetic information enough to design qPCR assays, we will sequence whole genomes of the FQ-resistant bacteria isolated in Activity 1. We will also use genome databases available through Infectious Disease units at the University of Minnesota and Minneapolis VA Hospital and public databases such PATRIC.

We will integrate FQ resistance qPCR assays into a high-throughput microfluidic qPCR protocol to simultaneously quantify multiple ARGs and genes that mediate FQ resistance. The qPCR assays will be validated and optimized using cultured bacteria isolates.

Outcome	Completion Date
1. qPCR assay development and analysis	November 30, 2019
2. Microfluidic qPCR analysis	February 29, 2020

Activity 3: *Comprehensive analysis of ARGs and FQ resistance in environmental water* **Budget: $25,000**

The high-throughput assays will be used to quantitatively detect ARGs and FQ-resistance genes. DNA samples obtained in Activity 1 and assays developed in Activity 2 will be used. We will use a statistical modeling to determine co-occurrence of FQ and other types of antibiotic resistance.

Outcome	Completion Date
1. Co-occurrences of ARGs and FQ resistance in environmental water samples	November 30, 2020

Activity 4: *Develop an information resource for tracking the spread of antibiotic resistance* **Budget: $170,000**

Using the resistance gene profiles obtained in Activity 3, we will develop an information resource that can be used to: 1) analyze potential health risks associated with the water samples; 2) identify a likely source of antibiotic resistance; 3) provide indicators and warnings about the spread of resistance. We will develop a user-friendly, cloud-based information resource for ease of access by local managers and regulators.

Outcome	Completion Date
1. Develop cloud-based computational infrastructure for a sequence-centric database	March 31, 2020
2. Deploy a user-friendly interface	June 30, 2020

III. PROJECT STRATEGY

A. Project Team/Partners

- *University of Minnesota*: Dr. Arkady Khodursky (Department of Biochemistry, Molecular Biology and Biophysics, and BioTechnology Institute, U of M) will manage and lead the project. Dr. Satoshi Ishii (Department of Soil, Water and Climate, and BioTechnology Institute) will supervise the development of the microfluidics assay. Dr. George Karypis (Department of Computer Science and Engineering) will provide technical assistance for informatics analysis. Dr. James Johnson (Department of Medicine, Division of Infectious Diseases) will provide genome data for FQ-resistant clinical isolates. Two graduate students will be hired: one for the assay development and data analysis; another for the development and deployment of computational resources. Undergraduate students will be hired for sample collection and analysis.
- *Minnesota Department of Health (No ENRTF Funding)*: Anita Anderson (Section of Drinking Water Protection) and Nancy Rice (Health Risk Assessment) will assist the team for sample collection and risk analysis.

B. Project Impact and Long-Term Strategy

The project will provide necessary resources for accurate, timely and targeted dissemination of surveillance data on antibiotic resistance in the state. The proposed approach will ensure that such dissemination of surveillance data, which is an essential component of efforts to combat the threat of resistance, will not be restricted to the scientific and medical community but will include all major stakeholders including the public as well as policy-makers and the state government.

C. Timeline Requirements

The proposed project will be completed in a two-year period. Samples will be collected and the assays will be developed and analyzed in the first 18 months. The development of the information resource will commence at the very beginning of the project and the resource will be fully operational by the project's end date.

2018 Detailed Project Budget

Project Title: ***Maximizing water safety against antibiotic resistance***

IV. TOTAL ENRTF REQUEST BUDGET 2 years

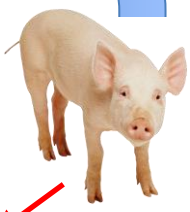
BUDGET ITEM	AMOUNT
Personnel:	
PI: Arkady Khodursky, Associate Professor (70% salary, 30% benefits); 8% FTE for two years; project supervision and coordination, data evaluation, information platform development, supervision of graduate students, project reporting.	$ 21,000
Co-PI: Satoshi Ishii, Assistant Professor (70% salary, 30% benefits); 8% FTE for two years; supervision of sample collection and qPCR assay development	$ 26,000
Graduate research assistants: 2 positions (55% salary, 45% benefits); 50% FTE for 18 months; perform microfluidic qPCR, data processing and analysis; development and deployment of the cloud-based information resource	$ 248,000
Undergraduate researchers: 2 positions (100% salary, 0% benefits); 100% FTE in summer for 5 months, 25% FTE during academic year for 10 months, sample collection, processing; data entry, quality assurance and pipeline testing	$ 26,000
Professional/Technical/Service Contracts:	
University of Minnesota Genomics Center: microfluidic qPCR (300 bacterial samples at $15/sample) and whole-genome sequencing of 10 reference persistent strains at 100x coverage	$ 11,000
Equipment/Tools/Supplies:	
Lab supplies (Membrane filters: 300 samples at $20/sample; DNA extraction kits for bacteria: 300 samples at $3/sample; Reagents for qPCR: (300 bacterial samples at $5/sample; other lab suppplies: 300 samples at $5/sample; $2,000 culture media, $1,000 antibiotics, and $2,000 plastic consumables). They are necessary to isolate FQ-resistant bacteria.)	$ 14,000
Travel:	
In-state travel to collect samples: (Approximately 3000 miles at .54/mile, meals at maximum $36/day)	$ 4,000
TOTAL ENVIRONMENT AND NATURAL RESOURCES TRUST FUND $ REQUEST =	$ 350,000

V. OTHER FUNDS

SOURCE OF FUNDS	AMOUNT	Status
Other Non-State $ To Be Applied To Project During Project Period:	N/A	
Other State $ To Be Applied To Project During Project Period:	N/A	
In-kind Services To Be Applied To Project During Project Period: The University of Minnesota does not charge the State of Minnesota its typical overhead rate of 53% of the total modified direct costs (graduate tuition and academic fringe are excluded).	$ 56,000	Secured
Past and Current ENRTF Appropriation:	N/A	
Other Funding History:	N/A	

Antibiotics

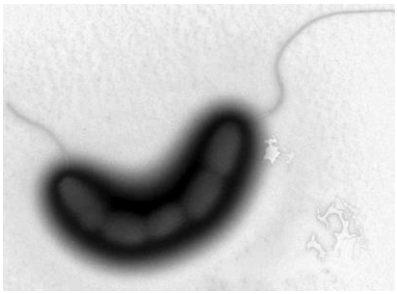
Hospitals

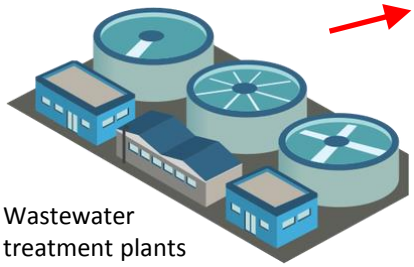


Farm animals

Emergence of

Antibiotics Resistant Bacteria (ARB)





Wastewater treatment plants

Improper manure handling

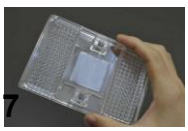
Antibiotics and ARB pollutions in MN water environments

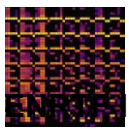
Many unknowns:

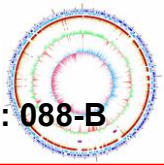
- What kind of ARB?
- How widely spread?
- Potential sources?
- Impact on public health?

Solution we propose:

- Newly designed DNA assays
- High-throughput test format
- Information resource to assist local managers and regulators







Project Manager Qualifications and Organization Descriptions

Arkady Khodursky

Arkady Khodursky is an Associate Professor in the BioTechnology Institute (BTI) and the Department of Biochemistry, Molecular Biology and Biophysics. Dr. Khodursky has over 15 years of experience working on antibiotic resistance, antibiotic mechanisms, functional genomics, statistics and data modelling. Dr. Khodursky designed first whole-genome bacterial DNA chip; he developed multiple applications of genomic technologies and discovered a key action mechanism of the quinolone antibiotics. His expertise in experimental design, data analysis and data modeling are critical for the success of this project. The Khodursky lab, which is located on St. Paul campus of the University of Minnesota, has all necessary equipment and resources for the proposed research, including full access to the microfluidic qPCR instrument and Illumina sequencers in the University of Minnesota Genomics Center.

Satoshi Ishii

Satoshi Ishii is an Assistant Professor in the BioTechnology Institute (BTI) and the Department of Soil, Water, and Climate (SWC) at the University of Minnesota. He joined the BTI and SWC in April, 2015. He has over 10 years of experiences on water quality microbiology. He has developed novel microfluidics tools to simultaneously quantify multiple pathogens and applied these tools to the risk assessment of water samples. The Ishii Lab is equipped with all the necessary items for the proposed research.

James R. Johnson

James R. Johnson is a Professor of Medicine in the Division of Infectious Diseases at the University of Minnesota and Minneapolis VA Hospital. Dr. Johnson is a leading expert on the ecology and conventional and molecular epidemiology of *E. coli* as an extraintestinal pathogen and colonizer of both healthy and compromised hosts. His strain collection at the Minneapolis VA Medical Center contains over 30,000 *E. coli* isolates from around the world, including more than 2,000 variants of epidemic strains.

George Karypis

George Karypis is a Professor of Computer Science, his research interests span the areas of data mining, bioinformatics, cheminformatics, high performance computing, information retrieval, collaborative filtering, and scientific computing. His research has resulted in the development of software libraries that will be used in the development of computational tools for tracking sources and the spread of antibiotic resistance.

Anita Anderson

Anita Anderson, P.E. is a Principal Engineer Supervisor with the Minnesota Department of Health Drinking Water Protection Section. Anita Anderson has 20 years of experience as a water supply engineer with the Minnesota Department of Health. Her primary area of expertise is surface water treatment, specializing in small systems. She is a registered professional engineer in Minnesota.

Nancy Rice

Nancy Rice is a Research Scientist with the Minnesota Department of Health Environmental Surveillance and Assessment Section. Nancy has been working since 2013 to research, develop, and implement quantitative microbial risk assessment (QMRA) for specific exposure scenarios, particularly water reuse.

Organization Descriptions

The University of Minnesota is the main research and graduate teaching institution in the state of Minnesota. Dr. Khodursky's lab, office and most essential supporting facilities are housed in the BioTechnology Institute on St. Paul campus of the University of Minnesota. Biotechnology Institute brings together more than 30 faculty members with expertise ranging from bioprocess technology to functional genomics, from synthetic biology to evolution.

The mission of the Minnesota Department of Health is to protect, improve, and maintain the health of all Minnesotans.