Environment and Natural Resources Trust Fund (ENRTF)
M.L. 2018 ENRTF Work Plan (Main Document)

Today's Date: February 20, 2018

Date of Next Status Update Report: January 31, 2019

Date of Work Plan Approval:

Project Completion Date: June 30, 2021

Does this submission include an amendment request? No

PROJECT TITLE: Mapping Antibiotic Resistance in Minnesota to Help Protect Environmental, Animal, and Human Health

Project Manager: Randall Singer

Organization: University of Minnesota

College/Department/Division: College of Veterinary Medicine, Department of Veterinary and Biomedical Sciences

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Location: Statewide

Total Project Budget: $750,000

Amount Spent: $0

Balance: $750,000

Legal Citation: M.L. 2018, Chp. xx, Sec. xx, Subd. xx

Appropriation Language:

I. PROJECT STATEMENT:

We will quantify and map antibiotic and antibiotic resistance gene (ARG) contamination in Minnesota waters and soils and then use this information to identify locations in need of mitigation to protect environmental, human, and animal health. The natural environment plays a key role in the emergence and spread of antibiotic resistance (AR). Watersheds, in particular, are recipients of antibiotics, antibiotic-resistant bacteria, and ARG released from human wastewater treatment plants, animal agriculture and aquaculture, crop production, and pharmaceutical manufacturing plants. The overall project goal is to mitigate effects of AR in Minnesota's natural environment on human, animal, and environmental health by:

- Developing an "antibiotic footprint" map of Minnesota's natural environment that predicts areas where antibiotics, resistant bacteria, and ARG are most likely to accumulate
- Quantifying concentrations of antibiotics and ARG at sites variably impacted by anthropogenic activities (ranging from pristine sites to areas with high impact)
- Validating the prediction map with the data collected across the state to develop a risk-based surveillance system that will aid in statewide AR mitigation efforts in the natural environment

We hypothesize that a predictive model developed for the state of Minnesota will identify "hotspots" for antibiotics, resistant bacteria, and ARG accumulation in the natural environment. This project will leverage past ENRTF-funded studies by our team members which have detected antibiotics and ARG in Minnesota lakes and rivers. The proposed work, which will produce tools for predicting areas sensitive to AR and for aiding in mitigation efforts, has never been attempted anywhere in the world and will place Minnesota as a leader in environmental antibiotic and AR detection, prevention, and response.

AR is one of the greatest public health challenges of our time. According to the Centers for Disease Control and Prevention, approximately 2 million people in the U.S. develop antibiotic-resistant infections each year, with more than 23,000 deaths. Our natural environment presents a reservoir for accumulated antibiotics and ARG that must be understood to minimize human health and ecological impact. Resistance genes of concern to human health have already been identified in Minnesota and in other U.S. waters and soil sediments, but it is unclear how these findings relate to modifiable factors (e.g., antibiotic prescribing, waste disposal) and adverse health outcomes. This proposal describes a comprehensive approach to the problem, linking human activity, environmental adulteration, and potential health threats.

In Minnesota, human, animal, and environmental health professionals have joined as the Minnesota One Health Antibiotic Stewardship Collaborative (http://www.health.state.mn.us/onehealthabx/), with a mission of promoting appropriate antibiotic use to reduce impacts of antibiotic-resistant pathogens on human, animal, and environmental health. As pledged in the *Minnesota Antibiotic Stewardship Five-Year Strategic Plan*, one goal of this nationally unique Collaborative is to understand the "footprint" of our collective antibiotic use on the natural environment and human health. Collaborative engagement ensures the benefits of sector-specific insight during project execution as well as dissemination of results to hundreds of stakeholders statewide.

II. OVERALL PROJECT STATUS UPDATES:

First Update January 31, 2019
Second Update June 30, 2019
Third Update January 31, 2020
Fourth Update June 30, 2020
Fifth Update January 31, 2021
Final Update August 15, 2021

III. PROJECT ACTIVITIES AND OUTCOMES:

ACTIVITY 1: Geospatial modeling of Minnesota's "antibiotic footprint"
Description: Antibiotic use in Minnesota's medical and agricultural sectors will be mapped to create an environmental "antibiotic footprint". These antibiotic use maps and geospatial models will then be used to predict the loading and persistence of antibiotics and ARG in the environment. Data collected in Activities 2 and 3 regarding the persistence and spread of antibiotic chemicals and ARG in the environment will be added to this dynamic map and will be used to validate the geospatial models. The geospatial models will be utilized to predict "hot spots" of antibiotic and ARG accumulation in Minnesota's natural environment, and can then serve as the basis for a functional risk-based surveillance tool.

ENRTF BUDGET: $ 291,602

Outcome	Completion Date
1. Estimation and mapping of antibiotic usage in Minnesota	June 30, 2019
2. Map-based modeling of the environmental fate of antibiotic compounds released from human and animal sources ("antibiotic footprint")	December 31, 2019
3. Comparison of model-predicted environmental "hotspots" of antibiotics and ARG with data collected during field sampling (Activities 2 and 3)	June 30, 2020
4. Use of "antibiotic footprint" to develop a risk-based surveillance tool and to identify opportunities for risk mitigation in medical, agricultural, and disposal sectors.	June 30, 2021

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ACTIVITY 2: Measure antibiotic concentrations in Minnesota's environment
Description: Widely used in medicine and agriculture, antibiotic chemicals are likely to be present in Minnesota's waters and soils due to release from wastewater treatment plants, spreading of manure on fields, and other release pathways into the environment. In conjunction with sampling for Activity 3, we will collect water, soil, river sediment, and manure samples at locations identified to likely be susceptible to contamination based on the initial mapping efforts of Activity 1. We anticipate collecting and processing 30-40 samples of each of the following sample types: municipal wastewater treatment effluents, rivers, lakes, soils, river and lake sediments, animal manures, and municipal wastewater sludges. Additional samples will be taken in year 3 to validate initial measurements and fill in gaps identified by continued mapping efforts. We will modify a method (developed by past ENTRF funding) to measure 20 antibiotic compounds in lake sediment for use on soil, water, and manure samples. Water samples will undergo solid phase extraction to concentrate the analytes. Solid samples will be extracted via an ultrasonic or accelerated solvent extraction, and then cleaned up and concentrated using the solid phase extraction step. Analysis will be performed using liquid chromatography-mass spectrometry to quantify the target antibiotics.
The information obtained will be used to assess the geospatial mapping prediction and also provide information to update the mapping efforts. Finding locations where antibiotic concentrations are elevated is important to identifying if and where management solutions/interventions are needed.

ENRTF BUDGET: $ 202,077

Outcome	Completion Date

1. Collection of soil and water samples	August 31, 2020
2. Antibiotic quantification in water samples	June 30, 2020
3. Antibiotic quantification in soil samples	June 30, 2021

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ACTIVITY 3: Quantify antibiotic resistance genes in Minnesota's environment
Description: Prior research has demonstrated that antibiotic resistance levels vary widely in the environment. In conjunction with sampling for Activity 2, we will collect water, soil, river sediment, and manure samples at locations identified to likely be susceptible to contamination based on the initial mapping efforts of Activity 1. We anticipate collecting and processing 30-40 samples of each of the following sample types: municipal wastewater treatment effluents, rivers, lakes, soils, river and lake sediments, animal manures, and municipal wastewater sludges. Additional samples will be taken in year 3 to validate initial measurements and fill in gaps identified by continued mapping efforts. Samples will first undergo DNA extraction and purification. Then, we will use a novel microfluidic quantitative polymerase chain reaction (MF-qPCR) to measure 24 different antibiotic resistance genes in lake sediment for use on soil and water samples. Total bacterial biomass will also be measured as 16S rRNA genes via conventional qPCR; this measurement will afford us some quality assurance/quality control for our methods (i.e., we have a general idea of the quantities of 16S rRNA genes per mass/volume that are found in the various sample types). This measurement will also afford us the opportunity to quantify the relative abundance of ARG types (i.e., number of ARGs per total number of bacteria).

ENRTF BUDGET: $ 256,321

Outcome	Completion Date
1. Collect soil, water, and sediment samples for quantification of ARG	August 31, 2020
2. DNA extraction/purification	December 31, 2020
3. Quantify ARG via a novel microfluidic method developed at UMN	May 1, 2021

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IV. DISSEMINATION:

Description:
This research project will have several key deliverables. First, Minnesota will become the first state to publish a comprehensive quantitative description of the fate of antibiotic-containing wastes within a state. Minnesota is a major leader in both agriculture and health care, as well as home to unique and diverse natural environments. A detailed description of the volume and distribution of antibiotic-containing waste in Minnesota will be a valuable resource for researchers looking to mitigate the presence of antibiotic resistance genes in waste water, sewage sludge, or livestock-sources fertilizer products, and to guide sampling for antibiotics, resistant organisms and resistance genes in high-risk locations, such as in areas at high risk for human exposure, and on agricultural soils used for vegetable production.

Second, we will generate datasets of antibiotic and ARG concentrations across the state. To our knowledge, the ability to link predictive spatial models to actual field collections has not been done before. We believe this will be the first study to investigate how the discharge of antibiotics is potentially (or is not) affecting the levels of resistance genes in the environment. This is information critical to protecting human and ecological health and may provide information relevant to antibiotic use and development. This study will reveal if additional treatment to remove antibiotics from wastewater or runoff is necessary or unnecessary in terms of proliferation of resistance genes. It will also identify the locations where maximum impact in terms of invested dollars/treatment can be made.

Finally, the knowledge and model obtained from this work can be used to target antibiotic stewardship activities (i.e., optimization of antibiotic use) and prescriber education, as well as to design future studies regarding antibiotic use behaviors and practices. Other practical uses of the environmental antibiotic footprint include prediction of the impact of "optimal antibiotic use" scenarios in human and animal health to understand expected benefits of antibiotic stewardship, crafting ecologically viable antibiotic stewardship messaging and interventions, informing disposal and processing of waste with antibiotic residues in high-risk areas, and estimating a timeline for changes in environmental antibiotic burden. Our project will define relationships between essential activities (e.g., healthcare, wastewater treatment, animal agriculture) and the maintenance and proliferation of AMR in Minnesota's natural environment. The long-term goal is to develop scientific and risk-based guidance in human, animal, and environmental health for the mitigation of AMR in the natural environment. The "footprint" methodology will also be useful to explore other biologically active chemicals in Minnesota's environment, such as hormones and endocrine disruptors. Results will be shared at local conferences, in open-access scientific publications, by publically available final report, and through the national reach of the Minnesota One Health Antibiotic Stewardship Collaborative.

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V. PROJECT BUDGET SUMMARY:

A. Preliminary ENRTF Budget Overview: See attached budget spreadsheet

Explanation of Capital Expenditures Greater Than $5,000: N/A

Explanation of Use of Classified Staff: N/A

Total Number of Full-time Equivalents (FTE) Directly Funded with this ENRTF Appropriation:

Enter Total Estimated Personnel Hours: 17,980	Divide by 2,080 = TOTAL FTE: 8.6

Total Number of Full-time Equivalents (FTE) Estimated to Be Funded through Contracts with this ENRTF Appropriation:

Enter Total Estimated Personnel Hours: 0	Divide by 2,080 = TOTAL FTE: 0

B. Other Funds:

SOURCE OF AND USE OF OTHER FUNDS	Amount Proposed	Amount Spent	Status and Timeframe
Other Non-State $ To Be Applied To Project During Project Period:			
	$	$	
Other State $ To Be Applied To Project During Project Period:			
University of Minnesota F&A	$ 497,655	$	Unrecovered F&A associated with research from U of MN (54%) during the three-year project period
Minnesota Department of Health	$18,728		In-kind contribution from Amanda Beaudoin, co-investigator, at 5% FTE for 3 years.
Past and Current ENRTF Appropriation:			
ML 2007-5L "Pharmaceutical and microbiological pollution in Minnesota's surface waters" (LaPara and Arnold)	$302,000		
ML 2010-5F "Evaluation of dioxins in Minnesota lakes" (Arnold)	$254,000		
ML 2011-5E "Assessment of Minnesota River antibiotic concentrations" (Wammer, LaPara, and Stoll)	$190,000		
ML 2013-5H "Antibiotics in Minnesota waters - Phase II Mississippi River" (Wammer, LaPara, and Stoll)	$203,000		
ML 2014-3C "Triclosan impacts on wastewater treatment" (LaPara and Donato)	$380,000		
ML 2014-3E "Antibiotics and antibiotic resistance genes in Minnesota lakes" (Arnold and LaPara)	$300,000		
ML 2016-4D "Assessing techniques for eliminating contaminants to protect native fish and mussels" (Wammer, Martinovic-Weigelt, Stoll, Schroeder)	$287,000		
Other Funding History:			
	$	$	

VI. PROJECT PARTNERS:

A. Partners receiving ENRTF funding

Name	Title	Affiliation	Role

B. Partners NOT receiving ENRTF funding

Name	Title	Affiliation	Role

Amanda Beaudoin	Director of One Health Antibiotic Stewardship	Minnesota Department of Health	Co-investigator

VII. LONG-TERM- IMPLEMENTATION AND FUNDING:

The major deliverable of this project will be a statewide risk-based surveillance system that will aid in statewide AR mitigation efforts in the natural environment. Future funding to maintain this project will be sought from various sources, including government agencies (state and federal). Because this project links environmental health with human and animal health, there will be a diversity of possible funding sources, and our team has had success in the past in obtaining funds from these sources. Finally, our team does a considerable amount of outreach activity, and the results of this project will be used in an outreach capacity to inform stakeholders about the risks associated with environmental AMR and opportunities that exist for mitigating risks associated with environmental AMR.

VIII. REPORTING REQUIREMENTS:

- **The project is for 3 years, will begin on 7/1/18, and end on 6/30/21.**
- **Periodic project status update reports will be submitted 1/31 and 6/30 day of each year.**
- **A final report and associated products will be submitted between June 30 and August 15, 2021.**

IX. SEE ADDITIONAL WORK PLAN COMPONENTS:

A. Budget Spreadsheet
B. Visual Component or Map

Visual is attached, and the following text explains the visual: Antibiotics, antibiotic resistant bacteria (ARB) and antibiotic resistance genes (ARG) can contaminate Minnesota's natural environment from a diversity of sources. As shown in the image, this project will build a tool for predicting environmental sites within Minnesota that are at risk of being contaminated with antibiotics and ARG. Steps include: 1) estimate the amount of antibiotic used in humans, animals and crops within Minnesota, 2) create a state map depicting areas of highest impact and loading, 3) use this antibiotic 'footprint' to determine sites for quantifying antibiotics and ARG in sediment and soil samples throughout the state, and 4) use the field data to validate and refine a geospatial model to improve its accuracy. Outcomes include: 1) Develop a tool to predict environmental contamination, and 2) Use the model to design risk-based mitigation strategies to protect health.

E. Research Addendum

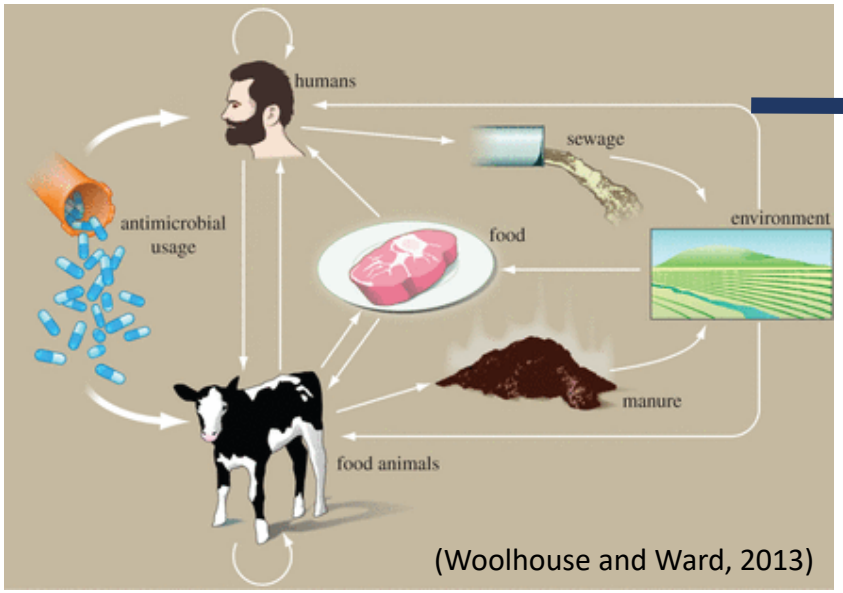
Attachment A:
Environment and Natural Resources Trust Fund
M.L. 2018 Budget Spreadsheet

Project Title: Mapping Antibiotic Resistance in Minnesota to Help Protect Environmental, Animal, and Human Health
Legal Citation:
Project Manager: Randall Singer
Organization: University of Minnesota
College/Department/Division: Department of Veterinary and Biomedical Sciences
M.L. 2018 ENRTF Appropriation:
Project Length and Completion Date: 3 years, June 30, 2021
Date of Report: February 20, 2018

ENVIRONMENT AND NATURAL RESOURCES TRUST FUND BUDGET	TOTAL BUDGET	AMOUNT SPENT	TOTAL BALANCE
BUDGET ITEM			
Personnel (Wages and Benefits)	$608,800		
Randall Singer, project manager; $24,850 Activity 1 (including 33.5% fringe; 4% FTE years 1-3)			
William Arnold, co-project manager; $23,867 Activity 2 (including 33.5% fringe; 4% FTE years 1-3)			
Timothy LaPara, co-project manager; $20,204 Activity 3 (including 33.5% fringe; 4% FTE years 1-3)			
Kristine Wammer, co-project manager; $5,219 Activity 2, $10,438 Activity 3 (including 8% fringe; 5% FTE years 1-3)			
Graduate student research assistant in Veterinary Medicine/UMN, data collection and analysis; $127,500 Activity 1 (61% salary, 39% fringe benefits; 50% FTE years 1&2, 25% FTE year 3)			
Geospatial analyst in Veterinary Medicine/UMN, develop and validate the geospatial models; $132,252 Activity 1 (82% salary, 18% fringe benefits; 61% FTE years 1-3)			
Graduate student research assistant in CEGE/UMN, sample collection, extraction, and analysis; $86,519 Activity 2 (58% salary, 42% fringe benefits; 50% FTE years 2&3)			
Post-doctoral research associate in CEGE/UMN, sample collection, extraction, and analysis; $118,034 Activity 3 (82% salary, 18% fringe benefits; 100% FTE for years 2&3)			
Undergraduates at CEGE/UMN, assist with sample collection and processing; $7,093 Activity 2, $14,187 Activity 3 (paid hourly)			
Undergraduates at UST, assist with sample collection, processing, and analysis; $12,879 Activity 2, $25,758 Activity 3 (paid hourly)			
Professional/Technical/Service Contracts	$58,950		
Analytical instrument time at UST; $2,700, Activity 3			
Analytical instrument time at UMN/Cancer Center; $38,250, Activity 2			
Analytical instrument time at UMN/UMGC; $18,000, Activity 3			
Equipment/Tools/Supplies	$53,250		
Supplies for sample collection, DNA extraction, and qPCR at UMN; $22,500, Activity 3			
Supplies for sample collection, DNA extraction, and qPCR at UST; $13,500, Activity 3			
Supplies for sample collection, extraction, and quantification of antibiotics at UMN; $17,250, Activity 2			
Travel expenses in Minnesota	$19,000		
Travel to collect water and soil samples (vehicle rental, meals, and hotel); $7,500 Activity 2, $7,500 Activity 3			
Travel to attend in-state conferences to disseminate results; $2,000 Activity 1, $1,000 Activity 2, $1,000 Activity 3			
Other	$10,000		
Open access publication charges; $5,000 Activity 1, $2,500 Activity 2, $2,500 Activity 3			
COLUMN TOTAL	**$750,000**		

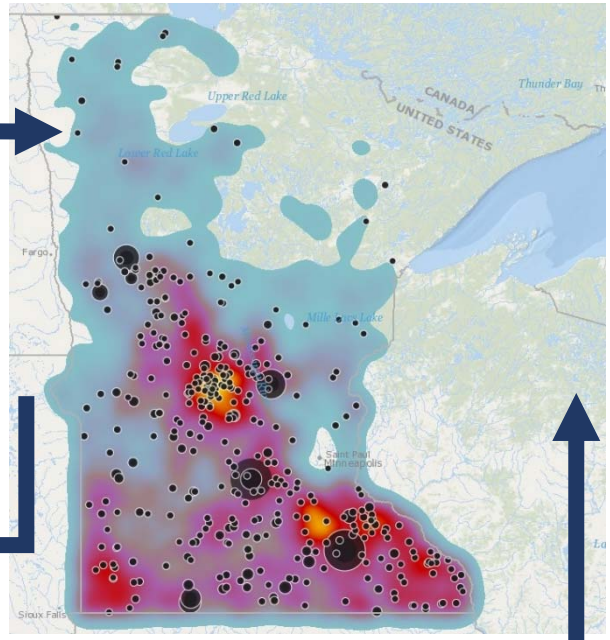
Mitigation of Antibiotic and Antibiotic Resistance Gene Pollution Requires Understanding the Sources of Contamination in Minnesota's Natural Environment

1. Estimate amount of antibiotics used in humans, animals and crops and resistance gene prevalence



(Woolhouse and Ward, 2013)

2. Build geospatial model predicting areas of highest impact and loading



3. Use geospatial model to identify sites where mitigation is needed

4. Use data collected in this project to validate model

Outcomes

- Develop a tool to predict environmental contamination with antibiotics and resistance genes
- Propose risk-based mitigation strategies
- Protect environmental, human, and animal health