2018 Project Abstract
For the Period Ending June 30, 2022

PROJECT TITLE: Mapping Antibiotic Resistance in Minnesota to Help Protect Environmental, Animal, and Human Health
PROJECT MANAGER: Randall Singer
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FUNDING SOURCE: Environment and Natural Resources Trust Fund
LEGAL CITATION: M.L. 2018, Chp. 214, Art. 4, Sec. 02, Subd. 04h as extended by M.L. 2021, First Special Session, Chp. 6, Art. 6, Sec. 2, Subd. 18

APPROPRIATION AMOUNT: $750,000
AMOUNT SPENT: $745,600
AMOUNT REMAINING: $4,400

<u>Sound bite of Project Outcomes and Results</u>

Our project mapped and quantified antibiotics and antibiotic resistance genes in Minnesota waters and soils. These findings are now used to target hotspots to better understand their fate and transformation in waterbodies. Ultimately, this information will be used for AMR mitigation strategies to protect environmental, human, and animal health.

<u>Overall Project Outcome and Results</u>

Antimicrobial resistance (AMR) threatens human, animal, and ecosystem health. Antibiotic use in hospitals, long-term care facilities, and animal husbandry operations (point sources) play a major role in AMR emergence. Discharges and runoff from these point sources which may include AMR and antibiotics enter the natural environment, especially waterbodies, in some cases after going through a treatment system at the point source itself or at a wastewater treatment plant. The project goals included a) developing an "antibiotic footprint" map of Minnesota's natural environment that would predict areas where antibiotics, resistant bacteria, and antimicrobial resistance genes (ARG) are most likely to accumulate; b) quantifying concentrations of antibiotics and ARG at sites variably impacted by anthropogenic activities; and c) validating the prediction maps with the data collected across the state to develop a risk-based surveillance system that will aid in statewide AMR mitigation efforts in the natural environment. To achieve the overall project goals, an iterative holistic approach was used which included sampling different environmental matrices at different spatial scales, and the use of diverse statistical and spatial methods to map and predict both antibiotics and ARG. The highest antibiotic concentrations were found near human populated areas, while ARG did not present any specific spatial pattern. The macrospatial approach identified hotspot areas of ARG and antibiotic contamination, and the microspatial approach revealed an influence of wastewater on ARG abundance. The maps and predictions created for waterbodies were useful to identify antimicrobial AMR and antibiotic hotspots areas throughout the state, while the maps created for soil can be used for targeted field surveillance of antibiotics. The environment plays a key role in the dissemination and persistence of AMR, which affects human, animal, and environmental health; therefore these findings are critical to continue developing mitigation strategies of AMR spread in Minnesota.

Project Results Use and Dissemination

This project has produced 2 peer-reviewed publications (Bueno, I., et al., 2021; and Bueno, I., et al., 2022) and there will be two others submitted soon summarizing the data for the 2020-2021 field seasons. This project has been presented at 9 (international and domestic) conferences both as poster and oral presentations, and during at least 2 teaching courses. Also, a graduate student used data from this project to conduct her Master's. One of the dissemination goals was to engage the general public at the state fair, but the COVID-19 pandemic halted that.

Environment and Natural Resources Trust Fund (ENRTF) M.L. 2018 ENRTF Final Report

Today's Date: September 28, 2022

Final Report

Date of Work Plan Approval: 06/05/2018

Project Completion Date: June 30, 2022

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

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Balance: $4,400

Legal Citation: M.L. 2018, Chp. 214, Art. 4, Sec. 02, Subd. 04h as extended by M.L. 2021, First Special Session, Chp. 6, Art. 6, Sec. 2, Subd. 18

Appropriation Language: $750,000 the second year is from the trust fund to the Board of Regents of the University of Minnesota to quantify and map antibiotic and antibiotic-resistance gene contamination in Minnesota waters and soils to identify locations in need of mitigation to protect environmental, animal, and human health. This appropriation is available until June 30, 2021, by which time the project must be completed and final products delivered.

M.L. 2021, First Special Session, Chp. 6, Art. 6, Sec. 2, Subd. 18. ENVIRONMENT AND NATURAL RESOURCES TRUST FUND; EXTENSIONS. [to June 30, 2022]

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:

Project Status as of January 31, 2019:

We have begun acquiring datasets for the mapping activities. We have obtained datasets regarding Minnesota's hydrography, land cover, soil, other environmental data, human population data, animal density data, and more. Antibiotic use datasets from humans and animals across the state have been started. We have also begun analyzing the samples collected thus far for antibiotic concentrations. We initially selected 20 antibiotics to analyze and we are currently considering an additional five. Finally, we have conducted our initial environmental sampling. Sample site locations were selected in order to provide a wide range of samples and were collected in 5 main areas in Minnesota: the metropolitan Twin Cities area, Mankato, Alexandria, Brainerd, and Ely. Water samples were collected from lakes, rivers, and WWTP effluents; sediment and soil samples were also collected. DNA was extracted and purified from cell suspensions following a Fast DNA kit protocol. Measurement of resistance gene quantities will begin in the next project period.

Second Update June 30, 2019
Amendment Request as of 06/30/2019: The ML 2018 04h project budget includes a line item "undergraduates at CEGE/UMN". This project requires a simple amendment request to remove "at CEGE/UMN" from cell A23.

Amendment Approved by LCCMR **7/19/2019.**

The mapping activities have expanded significantly since the prior report. Data from human and animal density, hospitals, wastewater treatment plant, and ethanol plant locations, as well as hydrography and landscape features have been included in the maps, and these maps have included data from Minnesota as well as neighboring states in order to account for the edge (or boundary) effects of the surrounding states that could influence the antibiotic loadings in the Minnesota (MN) natural environment. Using these improved maps, core areas (i.e. macro-geography) predicted to have the highest antibiotic loadings throughout the state of MN were chosen for sampling. The sampling strategy for each core area was based on the maps, as described in Activity 1. For the 2019 sampling, we also created a mobile data collection system that will allow all the field assistants to enter data in a standardized format using preloaded maps.

The samples that were collected in 2018 are currently being analyzed for the presence of 25 antibiotics. In addition, the quantities of the different resistance genes in the samples are currently being assessed for the 2018 samples. Field work for the 2019 season is beginning.

Third Update January 31, 2020
Amendment Request (01/31/2020): We are requesting to move $30,000 from Activity 1 (mapping) to Activity 2 (antibiotic measurement). While Activity 2 is proceeding smoothly, the graduate student working on the project will graduate by June 2020, and we wish to hire a postdoctoral researcher to complete the work for the last year of the project. Activity 1 has not required as much personnel as expected. A postdoctoral researcher is more expensive than the graduate student originally budgeted for Activity 2. Additional laboratory supplies are also needed for the remaining field sampling efforts. Overall, we would like to 1) repurpose the remaining funds for graduate student support in Activity 2 to support a postdoctoral researcher, 2) move $22,500 of graduate student support from Activity 1 to postdoctoral researcher support in Activity 2, and 3) move $7,500 of graduate student support from Activity 1 to laboratory supplies in Activity 2. This is detailed on the budget spreadsheet.

Amendment Approved by LCCMR **2/27/2020.**

Samples were collected in the summer of 2019 and are being analyzed for the presence of antibiotics and the quantities of the different resistance genes. Field work for the 2020 season is being planned. The mapping activities have provided some initial insights into predicting levels of antibiotics and antibiotic resistance genes in Minnesota's waterways. A peer-reviewed manuscript is being prepared that will present these initial data and demonstrate the approach we are using.

Fourth Update June 30, 2020

COVID-19 affected our project considerably. The field sampling season was changed (or eliminated) because of restrictions on movement and requirements for physical distancing. For this reason, much of the proposed field work was cancelled for 2020. A modified approach to sampling was created, but in limited format, and will be conducted during summer 2020. Much of the work conducted during this period was on the analysis of the data generated and the development of the spatial models. Peer-reviewed manuscripts are almost complete, with the first being submitted shortly.

Fifth Update January 31, 2021

Our 2020 field work was considerably modified due to COVID-19 restrictions. Despite the challenges, we were able to collect both water and sediment samples just from lakes around the Twin Cities during summer and early Fall. All the lakes sampled were impacted by human influence, but we were not able to sample in rural areas. The samples collected during 2020 will be processed by the end of spring 2021. We are finalizing two publications, which will be submitted to journals shortly, and we continue to collect new data for our final geo-spatial model.

Project extended to June 30, 2022 by LCCMR 7/1/21 as a result of M.L. 2021, First Special Session, Chp. 6, Art. 6, Sec. 2, Subd. 18, legislative extension criteria being met.

Sixth Update June 30, 2021
Amendment Request (06/30/2021): To complete the tasks in the project and because of changes in sampling and personnel priorities due to COVID-19, we are requesting the following changes in how funds are allocated. The budget spreadsheet has been changed accordingly.

- Personnel will decrease by $407 due to the following reasons. First, it has been difficult to hire undergraduates during COVID, as UMN and UST have both limited our ability to conduct field work and to use undergraduates for this work during the pandemic. We are providing additional funds to the postdoctoral researchers, who are currently working on the project and will be able to efficiently complete remaining tasks. We also moved the remaining funds for the graduate student in veterinary medicine to the geospatial analyst and Randall Singer (UMN) because the work is being completed by them; a graduate student will not be hired. By line item, these changes are as follows:
  - Increase Post-doc Personnel by $8,593 for UMN
  - Reduce Personnel for UST undergraduates by $9,000
  - Reduce Graduate student in veterinary medicine for UMN by $116,989
  - Increase Geospatial analyst in veterinary medicine by $110,000
  - Increase salary for Randall Singer by $6,989
- Reduce Professional/Technical/Service Contracts by $7,919. Rather than paying hourly charges to a centralized facility for these analyses, we are now able to run the antibiotic samples in our own laboratory, which allows more flexibility in timing and the ability to analyze samples in a more timely manner.
  - Reduce analytical instrument time at UMN/Cancer Center by $7,919
- Increase Equipment by $15,366. Much of this increase is related to the shift from using the centralized facility to performing the analyses in our own laboratory. For example, $10,000 is for the service contract/operation of a liquid chromatograph mass spectrometry system we were able to purchase for our laboratory for Activity 2. There are still costs associated with maintenance and operation of the instrument, but these are now categorized by UMN as maintenance. This change in budget line does not change the scope of this project. Maintenance/operation costs are apportioned based on usage of the instrument among various projects, and the amount requested reflects this usage.
  - Increase supplies for UMN Activity 3 by $2,500
  - Decrease supplies for UST Activity 3 by $3,000
  - Increase supplies for UMN Activity 2 by $15,866
- Reduce Travel by $7,040. The full amount of travel funds originally requested are not needed.
  - Reduce travel for UMN by $4,040
  - Reduce travel for UST by $3,000

Most of the work during the last six months has focused on dissemination of our project results to both scientific and lay audiences. For scientific audiences, we have submitted one manuscript with the results from 2018 and 2019, which is currently under review, and we are about to submit a second manuscript which covers the geospatial modeling work on vulnerability of soil to antibiotics. Further, we have presented our work orally at different venues, including the preliminary results from the 2020 fieldwork. For lay audiences, we are currently contributing to creating educational materials that will be used at the Minnesota State Fair 2021. We have also finalized the laboratory work for the 2020 samples (water and sediment) for both chemistry and antimicrobial resistance genes, and we are working on the data analyses. We are also currently working on the 2021 fieldwork season planning.

Amendment approved by LCCMR 9/13/2021

Seventh Update January 31, 2022

Between June 30 and January 2022, we have accomplished the following: we have conducted field work consisting of both water and sediment sampling in waterbodies throughout the state, as well as targeted soil sampling. We have also started to analyze these field samples in the laboratory for antibiotic concentrations and antibiotic resistance gene abundance quantification. We are currently finalizing the data analysis from the 2020 dataset and getting those results ready for publication. In terms of dissemination, we published our 2018-2019 results and have submitted another manuscript for publication. Additionally, we presented our project at one national conference, and we will be submitting our results to present at an international conference in 2022.

Final Update June 30, 2022 (Overall Project Outcomes and Results)

Amendment Request (06/30/2022): To complete the tasks in the project, there were some changes in how funds were allocated. The final budget had $4,400 remaining at the end of the project. The budget spreadsheet has been changed to match the final expenditures.

- Increase Personnel by $10,846 due to the following reasons. By line item, the changes are as follows:
    - Increase salary for Randall Singer by $3,204
    - Increase salary for Tim LaPara by $6,166
    - Reduce salary for Kristine Wammer by $10,438 (Activity 3)
    - Reduce salary for geospatial analyst by $9,261
    - Increase Graduate student in CEGE/UMN (Activity 2) by $36,271
    - Reduce post-doctoral research associated by $7,940
    - Reduce UMN undergraduates (Activity 2) by $7,093
    - Increase Personnel for UST undergraduates by $2,437
- Increase Professional/Technical/Service Contracts by $5,423.
    - Reduce analytical instrument time at UST by $2,700
    - Increase analytical instrument time at UMN/Cancer Center by $5,937
    - Increase analytical instrument time at UMN/UMGC by $2,186
- Reduce Equipment/Supplies by $9,616.
    - Reduce supplies for UMN Activity 3 by $4,000
    - Reduce supplies for UMN Activity 2 by $5,616
- Reduce Travel by $2,653. The full amount of travel funds originally requested are not needed.
    - Reduce travel to attend in-state conferences by $753 for Activity 1
    - Reduce travel to attend in-state conferences by $900 for Activity 2

    - Reduce travel to attend in-state conferences by $1,000 for Activity 3
- Reduce Other (Publication charges) by $4,000. The full amount of travel funds originally requested are not needed.
    - Reduce publication charges by $1,500 for Activity 2
    - Reduce publication charges by $2,500 for Activity 3

Amendment approved by LCCMR 1/13/23

Between January 31st, 2022, and June 2022, we continued to analyze the field samples collected from waterbodies throughout the state in 2021 in the laboratory to quantify antimicrobials and antimicrobial resistance genes (ARG). We finished the write up of the results from 2020 for publication. We have presented this project at several conferences and have published another manuscript in the peer-reviewed literature describing our maps of soil vulnerability to antimicrobial contamination in Minnesota. Importantly, the knowledge gained from this project has led to another ENRTF-funded project which we have already started. In the new project, we are sampling specific 'hotspot' sites at a microgeographic spatial scale so we can understand the impact of specific point sources on the dissemination of antimicrobials, and thus better assess mitigation strategies.

Antimicrobial resistance (AMR) threatens human, animal, and ecosystem health. Antibiotic use in hospitals, long-term care facilities, and animal husbandry operations (point sources) play a major role in AMR emergence. Discharges and runoff from these point sources which may include AMR and antibiotics enter the natural environment, especially waterbodies, in some cases after going through a treatment system at the point source itself or at a wastewater treatment plant. The project goals included a) developing an "antibiotic footprint" map of Minnesota's natural environment that would predict areas where antibiotics, resistant bacteria, and antimicrobial resistance genes (ARG) are most likely to accumulate; b) quantifying concentrations of antibiotics and ARG at sites variably impacted by anthropogenic activities; and c) validating the prediction maps with the data collected across the state to develop a risk-based surveillance system that will aid in statewide AMR mitigation efforts in the natural environment. To achieve the overall project goals, an iterative holistic approach was used which included sampling different environmental matrices at different spatial scales, and the use of diverse statistical and spatial methods to map and predict both antibiotics and ARG. The highest antibiotic concentrations were found near human populated areas, while ARG did not present any specific spatial pattern. The macrospatial approach identified hotspot areas of ARG and antibiotic contamination, and the microspatial approach revealed an influence of wastewater on ARG abundance. The maps and predictions created for waterbodies were useful to identify antimicrobial AMR and antibiotic hotspots areas throughout the state, while the maps created for soil can be used for targeted field surveillance of antibiotics. The environment plays a key role in the dissemination and persistence of AMR, which affects human, animal, and environmental health; therefore these findings are critical to continue developing mitigation strategies of AMR spread in Minnesota.

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: $ 274,292

Summary Budget Information for Activity 1:

ENRTF Budget:	**$ 274,792**
Amount Spent:	**$ 274,151**
Balance:	**$ 641**

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

Activity 1 Status as of January 1, 2019:
The initial focus of Activity 1 was to acquire and curate data from the state of Minnesota. Specifically, the datasets obtained thus far include: hydrography, land cover, soil, environmental data; point source data (such as wastewater treatment plant locations, farms, feedlots, fisheries, ethanol plants), human population data and animal density data. These datasets have been acquired from both public sources and from private sources provided through the collaboration with U-Spatial at the University of Minnesota. The data have been entered into an ArcGIS project, and initial maps have been generated. Antibiotic use datasets from humans and animals across the state have been started. Data on human antibiotic use are being estimated using published reports and datasets that are becoming available through the Minnesota Department of Health. For food animals, we are in the process of estimating antibiotic use for the different commodities in Minnesota using published reports and data obtained from producers. For companion animals, antibiotic use data are being estimated using published reports at the national level. There are multiple new data collection initiatives starting in Minnesota, and we hope to have state-specific companion animal data to incorporate into the model by the end of the three-year project period.

Activity 1 Status as of June 30, 2019
Data acquisition, curation, and refinement of relevant datasets has continued to improve the initial maps. In addition to what had already been collected, data from Minnesota's neighboring states (IA, WI, ND, SD) relevant to the outcome of the project was obtained. These data include human and animal density, hospitals, wastewater treatment plant, and ethanol plant locations, as well as hydrography and landscape features. The project team considered it necessary to collect these data in order to account for the edge (or boundary) effects of the surrounding states that could influence the antibiotic loadings in the Minnesota (MN) natural environment.

Using the improved maps, core areas (i.e. macro-geography) predicted to have the highest antibiotic loadings throughout the state of MN were chosen (**Fig. 1**). Within these core (or macro-geographic) areas, specific sampling sites (micro-geography) were selected based on information such as discharge location of wastewater effluent and waterbody size and depth, combined with feasibility to access the sites for sampling. In addition, a sampling strategy was designed for each one of the sampling sites. This sampling strategy incorporated a gradient consisting of the collection of at least one sample upstream from the point source effluent (for example a wastewater discharge pipe), and several downstream samples collected at different distances from the point source effluent location, with an overall distance between the most upstream site and the furthest downstream sites of no more than 2 miles to avoid the influence of other potential sources of antibiotics. An example of the sampling gradient is shown in **Fig. 2**. The sampling sites within the core areas were discussed with the entire team and refined based on the team's feedback.

In preparation for field work during summer 2019, a Collector map for ArcGIS was created using the ESRI™ Collector App for mobile devices (**Fig. 3**). This map was shared with the entire team and with the field workers so that they can gather relevant information in the field (GPS coordinates, water measurements, temperature, etc.) into a single collective map. After field work, these data can be directly uploaded into the ArcGIS project. Results obtained from the samples collected during the Summer 2019 throughout the state will be used to update the ArcGIS project and to obtain improved predictions of antibiotic loadings in the natural environment.

Figure 1. This simplified map depicts core areas (i.e. macro-geography) throughout the state of Minnesota predicted to have higher antibiotic loadings based on the available datasets (open purple circles represent the core areas). The blue lines and polygons represent waterbodies.

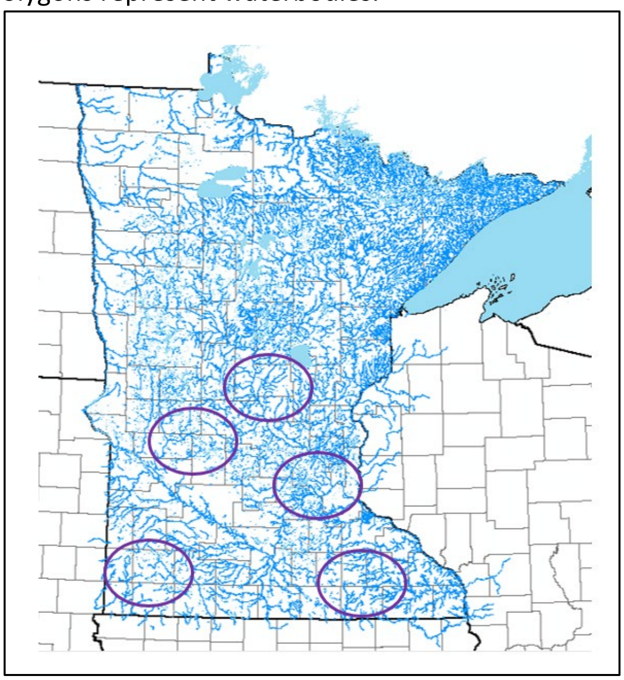


Figure 2. Within the core area depicted in red, this is an example of the sampling gradient designed for specific waterbodies. The blue stars represent sampling locations: U1 represents the upstream location from the wastewater treatment plant effluent (WWTP); D1 through D3 represent downstream locations. The green symbols depict access to the lake and creek.

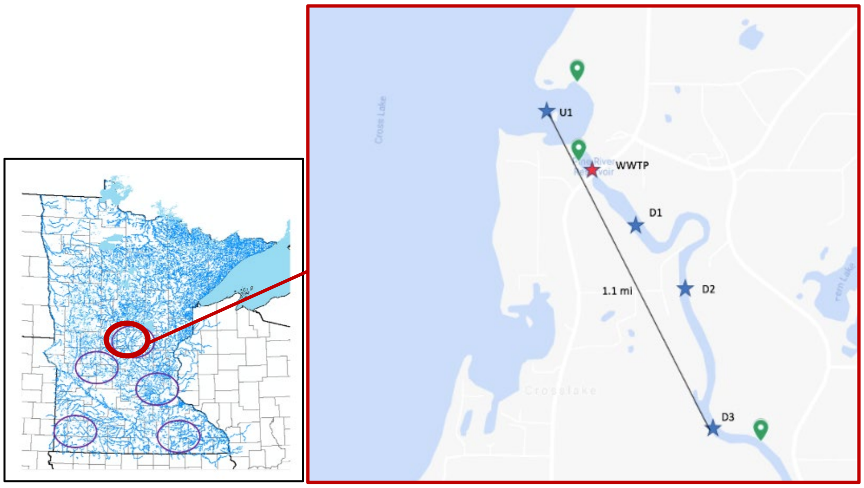
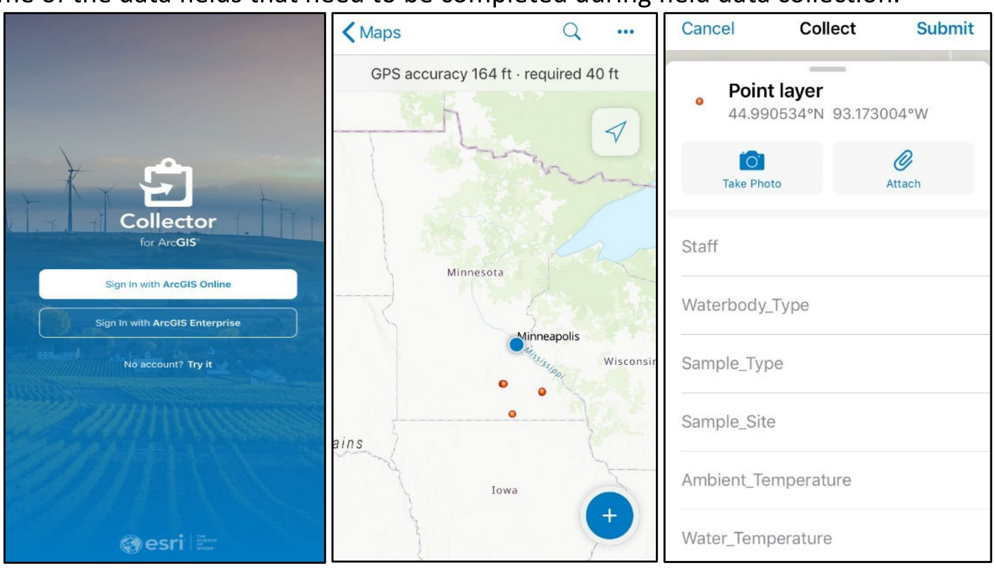


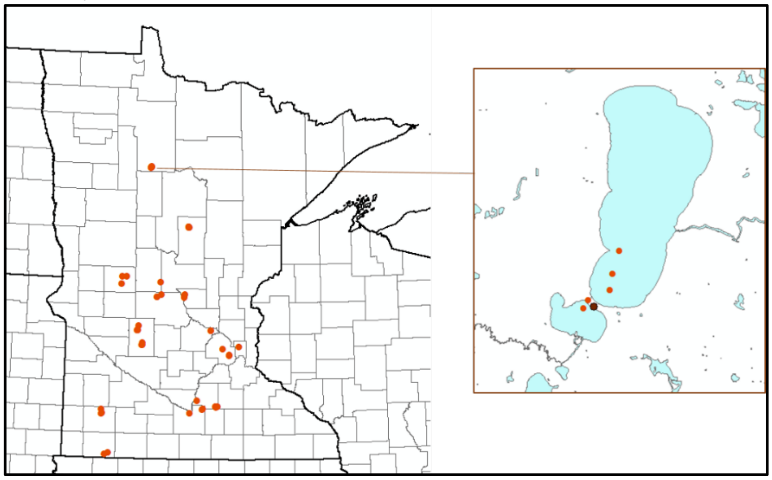
Figure 3. Screen captures of the Collector App for ArcGIS in a mobile device. The picture on the left is the login screen for the App; the middle picture represents the shared map for the team to use in the field (the orange dots depict sampling locations; the blue circle with the plus sign is where to click to add data); the picture on the right shows some of the data fields that need to be completed during field data collection.



Third Update January 31, 2020

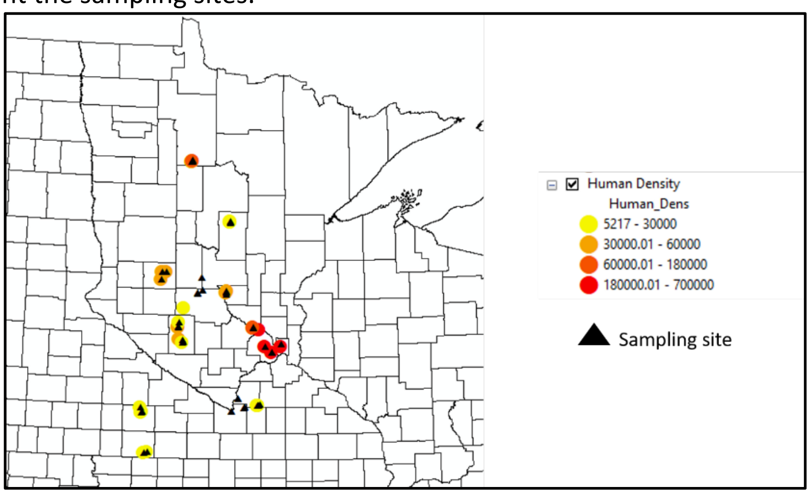
The final specific field sites for the summer 2019 are depicted in **Fig 4**. The final sites ended up being a combination of the sampling strategy we designed plus feasibility to access those sites. In locations where there was discharge from a wastewater treatment plant, samples were taken at several spots upstream and downstream from the plant, as can be seen in **Fig 4**.

Figure 4. Final field sites for the summer 2019 (orange dots). The inset map (right) depicts all the points (in orange) that were sampled within 1 site when there was an effluent from a wastewater treatment plant at that site (brown dot in the inset map).



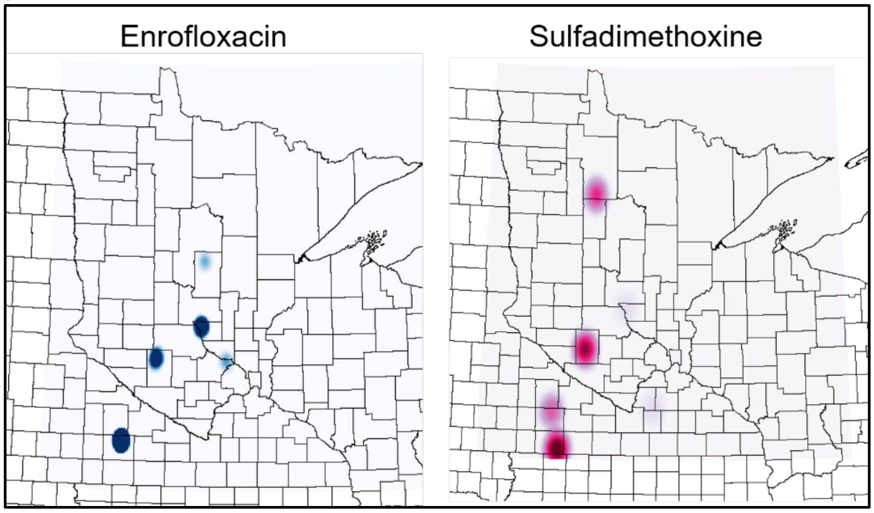
Data from the samples that have been processed so far have been curated and are being analyzed. Potential variables influencing the antibiotic concentrations and antibiotic resistance genes found in the field have been gathered, mapped, and we are currently conducting in depth geo-statistical analyses. These variables include physical-chemical parameters (pH, water temperature, ambient temperature, dissolved oxygen) measured at the field sites, distance to the nearest wastewater treatment plant, as well as human and animal density around the sampling sites. An example of how we are mapping these variables can be seen in **Fig 5**.

Figure 5. Human Density around the 2019 sampling sites. Red areas are the ones with more human density. The black triangles represent the sampling sites.



Preliminary results of the geo-statistical analyses are showing areas with higher specific antibiotic concentrations among the sampling sites. As an example, **Fig 6** shows two maps depicting two different antibiotics measured in water samples in the 2019 field campaign.

Figure 6. Enrofloxacin (left) and sulfadimethoxine (right) concentrations after conducting a kernel density analysis that is starting to show where we might see ‘hotspots’ of certain antibiotics throughout the state based on our field sampling.



Fourth Update June 30, 2020

Spatial and statistical analyses have been conducted for the antibiotic concentrations and antibiotic resistance gene results from both water and sediment samples from 2018 and 2019 field seasons. Currently, we are working on a scientific publication to report the results from the water samples. Briefly, we found that the locations with the highest antibiotic concentrations from our sample sites throughout MN were mostly found at or near densely human populated areas (Lake Harriet, Lake Owasso, Medicine Lake, Otsego creek). Further, the specific compounds that had the highest concentrations were antibiotics that are commonly prescribed in human medicine, such as quinolones and macrolides (we have obtained these data from the Minnesota Department of Health). Based on our results and using geospatial methods, we were able to estimate the concentrations of key antibiotics in areas that were beyond the sites where we sampled. We also found an effect of wastewater discharge increasing the abundance of a beta-lactamase gene, which can confer resistance to antibiotics such as penicillin and cephalosporins. Overall, the results show that human density and antibiotics used in human medicine may have more of an influence on the antibiotics and genes that we find in waterbodies in Minnesota than other sources. However, our dataset is still small at this point in time, and thus our predictions have measureable uncertainty. We are also finalizing a spatial model for the state of MN that will identify areas of soil vulnerability to antibiotics. To inform the modeling process, a targeted literature review focused on the chemistry of specific antibiotic compounds was conducted from February until June by undergraduate students from the University of St. Thomas (PI: Dr. Kristine Wammer).

An additional activity that took place during this period of time was to design the summer 2020 field sampling. Due to the restrictions related to the COVID-19 pandemic, the field sampling for the summer 2020 had to be drastically modified. These restrictions included the inability to use the necessary equipment to collect meaningful and comparable samples with 2019 summer sampling (in fact we could not collect sediment samples, only water), as well as the limitation of not being able to travel beyond the Twin cities area to sample. Thus, we selected 20 random lakes with public access and with at least two docks per lake in order to collect a composite water sample per lake. In addition to the sample, students collected physical-chemical parameters with the allowable equipment. We built a new ArcGIS online map for the 2020 summer sampling that connected directly to the ESRI App collector of the student’s cellphones in the field so that the data can be uploaded into the ArcGIS online directly for analyses.

Fifth Update January 31, 2021
During the summer/early Fall 2020, we participated in the collection of sediment samples from lakes around the Twin Cities. This assistance provided support to the rest of the project team, as undergraduates were unable to assist with the field work (sediment sampling) due to COVID restrictions.

We collaborated with Dr. Ana de la Torre (Centro de Investigación en Sanidad Animal, CISA-INIA, Spain) to build a geo-spatial soil vulnerability model for the state of Minnesota. The model identified the areas of Minnesota with the highest soil vulnerability to the most widely prescribed antibiotics. We are currently finalizing the manuscript for this model and we will also be submitting it to be presented at a conference during 2021. We are also about to submit the manuscript that contains the results from the data analyses of the 2018-2019 field sampling.

We continue to gather new data on antibiotic use. On the animal side, Dr. Singer was part of a special issue published in the journal Zoonoses and Public Health titled "Special Issue: Antimicrobial Use Data Collection and Reporting'. Dr. Singer, along with his collaborators, released data on estimates of on-farm antimicrobial use for different animal commodities. On the human side, Dr. Beaudoin is actively working with the Centers for Disease Control and Prevention (CDC) to acquire data on antibiotic prescriptions for Minnesota. These new datasets will be incorporated into our final geospatial model.

Sixth Update June 30, 2021
We have submitted a manuscript for publication with the results from the work we conducted in 2018 and 2019. This manuscript is currently under review in the Scientific Reports Journal (https://www.nature.com/srep/). We are now finalizing an improved version of the geospatial model for soil vulnerability to antibiotics in Minnesota and this manuscript will be submitted to the Environmental International Journal (https://www.journals.elsevier.com/environment-international) in the upcoming weeks. We have also been working on mapping the results from the 2020 fieldwork. Finally, we are also working on the 2021 field work season planning which includes reviewing previously created maps and results, as well as preparing a new map for 2021 with the ESRI App collector.

Seventh Update January 31, 2022
Our results from the 2018-2019 work were published in the *Scientific Reports* Journal in September 2021: "Bueno, I., Beaudoin, A., Arnold, W. A., Kim, T., Frankson, L. E., LaPara, T. M., ... & Singer, R. S. (2021). Quantifying and predicting antimicrobials and antimicrobial resistance genes in waterbodies through a holistic approach: a study in Minnesota, United States. Scientific Reports, 11(1), 1-15": https://www.nature.com/articles/s41598-021-98300-5. We also have "under review" another peer-reviewed publication in the *Science of the Total Environment* Journal with our models of antimicrobial soil vulnerability in Minnesota. We are also assisting the rest of the team with the 2020 data analyses and getting that ready for publication.

Final Report Summary
Since the last progress report, we have been finalizing the spatial and data analyses for the 2020 dataset from waterbodies around urban areas and have written up the results for publication. Our work mapping soil vulnerability to antimicrobial contamination in Minnesota was published: *"Bueno, I., Rodríguez, A., Beaudoin, A., Arnold, W. A., Wammer, K. H., de la Torre, A., & Singer, R. S. (2022). Identifying the spatiotemporal vulnerability of soils to antimicrobial contamination through land application of animal manure in Minnesota, United States. Science of the Total Environment, 832, 155050. DOI: 10.1016/j.scitotenv.2022.155050".* The results from this work can be used in the future to guide targeted soil sampling in the state of Minnesota to quantify antimicrobials. The 2021 field samples are being processed in the laboratory, and as soon as they are finished, we will analyze that dataset for publication as well. The analyses will be conducted by current research staff from Dr. Randall Singer's lab with the help of collaborators. The publication cost will be covered by departmental funds.

To summarize Activity 1, antibiotic use as well as antibiotic residues and antimicrobial resistance (AMR) sources in Minnesota's medical and agricultural sectors were mapped. Data collected from Activities 2 and 3 were used to predict antimicrobial concentrations and antimicrobial resistance genes (ARG) abundance throughout the state. These maps mostly focused on waterbodies, but we also mapped the vulnerability of soil to antimicrobial contamination in Minnesota. The models and maps created were used to highlight areas in the state that might be potential 'hotspots' of antibiotic and ARG accumulation. One of the main lessons learned from this activity was the use of different spatial scales (macro and micro) to better understand the question at task. The macro scale focused on the state at large, while the micro scale focused on specific 'hotspots' to assess more localized antibiotic and ARG dynamics. In fact, this advancement led to specific 'hotspots' being currently evaluated with the new ENRTF-funded project titled "Microgeographic impact of antibiotics released from identified hotspots". The information obtained from both projects will serve as the basis to establish mitigation strategies to minimize AMR and antimicrobial dissemination in Minnesota's natural environment.

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: $ 238,231

Summary Budget Information for Activity 2:

ENRTF Budget:	**$ 238,231**
Amount Spent:	**$ 235,802**
Balance:	**$ 2,429**

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

Activity 2 Status as of January 1, 2019: Initially 20 antibiotics were selected to analyze for in the environmental samples. An additional 5 are being analyzed to determine the feasibility of adding them to the suite of analytes. These antibiotics were chosen based upon their use in Minnesota. In conjunction with sampling for Activity 3, eighteen water samples from across Minnesota were collected in the summer months of 2018. They are in the process of being analyzed for the presence of antibiotics. Student training regarding the sediment extraction step for analyzing antibiotics in sediments is also currently occurring.

Activity 2 Status as of June 30, 2019: In total, 25 antibiotics were chosen for analysis on the samples collected in this project. Water samples from the summer months of 2018 were analyzed for antibiotics as well as wastewater effluents from 8 Metropolitan Council wastewater treatment plants. Sampling this summer for antibiotics has begun for both water and soil samples and will continue throughout the summer.

Third Update January 31, 2020

During the summer of 2019, 36 water samples and 38 sediment samples were collected. All of the water samples have been processed and analyzed for antibiotics. Half of the sediment samples have been processed and analyzed for antibiotics. The rest of the sediment samples should be completed in the next 1.5 months.

Fourth Update June 30, 2020

Analysis was completed for water and sediment samples collected during 2019. The data were used to evaluate the impacts of land use and presence of wastewater treatment plants on the identities and quantities of antibiotics present. The data collected comprised the MS Thesis of Lara Frankson, and the data are being used in the modeling efforts of Activity 1. The COVID 19 pandemic disrupted planned sampling from April until July. With the physical distancing and travel restrictions that are still in place, sampling has been limited to collection of water samples from lakes and wastewater treatment plants in the Minneapolis/St. Paul Metro area. We have sampled nine wastewater treatment plants and 20 lakes, and we are planning additional water sampling through October. Soil and sediment sampling will not be possible until social distancing/travel restrictions are relaxed due to the need to have multiple people in a boat to perform this sampling.

Fifth Update January 31, 2021

In August-October 2020, additional water samples were collected from lakes and creeks in or near the Minneapolis/St. Paul Metro (MSP) area. They include 10 lakes in (sub)urban areas (mainly within Ramsey County), and one lake with limited impact (Lake Itasca, which could act as a reference to assess the background levels of antibiotics), and 4 creeks (for some, multiple samples were taken along the creek to evaluate small-scale geospatial distributions). Sediment samples (30 in total) were taken from the 10 lakes plus the 20 lakes mentioned in the Fourth Update. To date, solid-phase extraction has been performed on all the water samples followed by LC-MS/MS analyses, while the sediment samples are being processed. It is expected this will be completed over the winter. The next stage will focus on (1) collaborating with Minnesota Department of Agriculture to obtain samples from a larger array of creeks in the MSP area and around MN state to fill in the map, (2) analyzing the data of antibiotic concentrations in lakes/creeks and comparing that with land use data to identify the primary antibiotic sources due to human activities, and (3) developing a new method based on Orbitrap LC-MS to allow quantification of all the 25 target antibiotics in one assay, which would be more time- and cost-effective than the current method based on LC-MS/MS.

Sixth Update June 30, 2021

The samples (39 lake/creek water samples plus 30 lake sediment samples) collected in August-October 2020 have all been processed and analyzed. Preliminary results showed that 23 out of the 25 antibiotics were detected in the 39 water samples, with detection frequencies of the classified antibiotics declining in the order of sulfonamides (95%) > macrolides (79%) > beta-lactams (67%) > tetracyclines (28%) > fluoroquinolones (26%). In general, total antibiotic concentrations were generally higher at urban sites, with very high concentrations of certain antibiotics detected in several urban lakes; for selected creeks/rivers, certain antibiotic classes (e.g., sulfonamides) exhibited higher concentrations at downstream sites compared to upstream sites. The spatial distribution of targeted antibiotics in urban lake sediments seemed to be different from that in their water columns – lakes with high-level antibiotics in the water columns did not necessarily exhibit high concentrations of antibiotics in the sediments based on our observations.

Ongoing work is focusing on extracting information of anthropogenic activities (e.g., land cover, population, domestic/industrial/hospital wastewater discharge locations, etc.) within the catchment areas of each water body. Statistical tools will be applied to investigate their relations with the measured antibiotic data. In addition, the antibiotic data of the 30 (sub)urban lakes (for both water and sediments) will be analyzed in conjunction with the antimicrobial resistance gene (ARG) data (for sediments) to generate a whole profile of antibiotics and ARG in the urban-area water ways.

Meanwhile, a new method has been developed to allow analyses of all the 25 target compounds and 10 surrogates/internal standards in one injection on an Agilent LC-MS/MS instrument. Further method optimization will be conducted to enhance sensitivity of several compounds with low responses (such as beta-lactams).

Seventh Update January 31, 2022

We are now focusing on analyzing the data obtained for samples of summer 2020 (antibiotic and ARG concentrations in lake/creek water and sediment samples collected primarily in the MSP (sub)urban area). Statistical analysis for antibiotics in water samples show that increasing percentages of open water, wetland, and forest contributed to decreasing number of detected antibiotics, as well as to decreasing concentrations of certain antibiotics, e.g., sulfamethoxazole and total sulfonamides (but not for every compound or every class of them); while percentage of urban/impervious areas, population density, and number of wastewater discharge points and hospitals, positively correlated with number of detected antibiotics and concentrations of selected compounds. Livestock density also seems to positively impact antibiotic occurrences and concentrations in surface water; however, since our sampling locations were primarily within the (sub)urban areas leading to only a few sites with livestock density > 0, the results only show weak correlation with poor significance. In general, our results support the hypothesis that antibiotic occurrences and concentrations in surface water environments are affected by landcover gradient and human activities with statistical significance. Similar analyses have also been conducted for antibiotic and ARG occurrences and concentrations in sediment samples, but no clear trends with statistical significance were found for that data, implying the complexity of antibiotic accumulation and antibiotic resistance development in surface water sediments. The results above are being organized into a manuscript, which will be submitted to a peer-reviewed journal in January/February.

In addition, we have performed another sampling campaign in the summer of 2021 (July to early October). We collaborated with the Department of Agriculture (MDA), MN, and collected water and/sediment samples from ~50 rivers/creeks around the whole state. For these samples, we applied two extraction methods in parallel: (1) the existing method using HLB cartridges; and (2) a multi-layer self-packed cartridges containing mixed sorbents, which will hopefully improve extraction efficiency and matrix disruption. We will compare the performance of the two methods and apply the established statistical approaches to the 2021 results, to validate whether the trends found in urban area applies to a state-wide range of landcover gradient and human activities.

Final Report Summary

As per new information since the last progress report, we have completed all the data analyses for the results of samples obtained in the 2020 field season. Results of detailed statistical analyses support our preliminary findings in the last update: for water samples, the number of detected antibiotic types and concentrations of certain antibiotics (e.g., sulfonamides) exhibited significantly positive correlations with indicators representing urbanization (e.g., urban percentage, population density, number of wastewater discharge points;) and negative correlations with undeveloped land indicators (e.g., forest;). Further, principal component analysis (PCA) showed that locations of sampling sites on PCA score plots based on antibiotic concentrations in water samples shifted with increasing urbanization, and there was significant difference between groups of sampling locations with low (≤ 30%), moderate (30%–60%), and high (≥ 60%) urban percentages. Antibiotics in corresponding sediments exhibited a different geospatial pattern in urban surface waters, and no associations with anthropogenic factors. Meanwhile, we are finalizing analysis of the water and sediment samples obtained in the 2021 field season. A new solid-phase extraction method using multi-layer mixed-sorbent cartridges was developed. Although this method has difficulty in getting satisfactory recovery for fluoroquinolone antibiotics, it had better recoveries for other classes of antibiotics (e.g., sulfonamides) and largely improved matrix effect compared with our old method using HLB cartridges. Using the 2021 dataset in combination with the 2020 dataset, a model for prediction of antibiotic occurrences in Minnesota surface waters using the anthropogenic indicators identified above will result. These analyses will be led by Dr. Randall Singer's research staff in collaboration with Dr. Bill Arnold's group and the publication cost will be covered by their Department funds.

To summarize Activity 2, we have sampled water and sediment samples in different sites throughout the state as well as at different times and have quantified different antimicrobials from these samples. To increase our sample size, we collaborated with the Minnesota Pollution Control Agency and the Minnesota Department of Agriculture. We initially chose the specific antibiotics based on the compounds used in humans and animals in the state. We have been able to improve the precision of our laboratory methods over time, being now more efficient at quantifying several antibiotics at the same time. Much of what we have learned with this project is already being used in the newly funded ENRTF project evaluating antibiotics in water at a microgeographic scale.

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: $ 236,977

Summary Budget Information for Activity 3:

ENRTF Budget:	**$ 236,977**
Amount Spent:	**$ 235,647**
Balance:	**$ 1,330**

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

Activity 3 Status as of January 1, 2019: Initial work on Activity 3 was focused on the students' learning techniques involving sampling, processing, and data analysis in preparation for the main sampling effort that will be directed by the mapping efforts in Activity 1. Sample site locations were selected in order to provide a wide range of samples and were collected in 5 main areas in Minnesota: the metropolitan Twin Cities area, Mankato, Alexandria, Brainerd, and Ely. Water samples were collected from lakes, rivers, and WWTP effluents; sediment and soil samples were also collected. Water samples were collected using a Rexeed membrane filter via a peristaltic pump powered by a generator. Samples were taken near the middle of the lake or river from a canoe in order to sample past the photic zone. Wastewater treatment effluent samples were collected using acid-washed glass bottles. Sediment samples were collected using a Ponar or Ekman dredge. Soil samples were collected in autoclaved 1.5 mL centrifuge tubes. Samples were kept on ice and processed within 24 hours. For water samples, membrane filters were backflushed and flocculated to produce a pellet. DNA was extracted and purified from cell suspensions following a Fast DNA kit protocol. Although, as described above, MF-qPCR will be used for the majority of gene quantification, conventional qPCR was used to quantify two genes: 16S rRNA genes and tetA.

Activity 3 Status as of June 30, 2019
In support of the work described in Activity 1, students have investigated and scouted locations for sample collection. In addition, new students to the team have been trained on sample collection protocols and sample processing, and equipment has been obtained to allow scaling up efforts to allow sample processing at both the University of Minnesota and St. Thomas, which will help with efficiency. With all sites selected and a plan in place for Summer 2019 sampling, students have just begun to obtain water, sediment, and soil samples. This effort will continue throughout the summer, and conventional qPCR will again be used to quantify 16S rRNA genes and *tet*(A) genes.

Third Update January 31, 2020
Water and sediment samples were also collected, in parallel with Activity 2, to measure antibiotic resistance genes. Metagenomic DNA has since been extracted and purified from these samples, which served as template to quantify six different genes by real time PCR and to quantify 24 different genes by microfluidic PCR (the same six genes targeted by real time PCR plus 18 additional genes).

Fourth Update June 30, 2020
Analysis continued for the DNA that was extracted from the samples. Because of COVID-19, the equipment needed for the DNA analysis was unavailable for most of this project period. Hopefully the equipment for microfluidic PCR will become available again in summer 2020.

Fifth Update January 31, 2021
An additional 30 sediment samples were collected during the summer of 2020. DNA has been extracted and purified from these samples and quantification of antibiotic resistance genes is on-going. Progress on this activity has been severely limited because of the COVID pandemic and the University's rules for safety.

Sixth Update June 30, 2021
Thirty lake sediment samples were collected in August-October 2020 concomitant to the samples collected as part of Activity 2. Metagenomic DNA was extracted and purified from these 30 lake sediment samples and used as template to quantify total bacteria (16S rRNA genes), class 1 integrons (*intI1*), and several genes encoding resistance to tetracycline (*tetA*, *tetX*, and *tetW*) via real time polymerase chain reaction (PCR). Preliminary results showed that *tetX* and *tetW* genes were negative in all the samples, but *intI1* (56% of samples) and *tetA* (52% of samples) were frequently detected in sediment samples.

Seventh Update January 31, 2022
During the Summer of 2021, 19 sediment samples and 23 water samples were collected throughout the state. DNA was extracted from all the 42 samples collected in 2021 as well as 30 sediment samples collected in 2020. Samples collected in 2020 were processed again from DNA extraction since PCR inhibition was suspected with some samples. Total bacteria (16S rRNA genes), three integrase genes (*intI1*, *intI2*, and *intI3*), and 20 antibiotic resistance genes (*ampC*, *blaOXA*, *blaSHV*, *ereB*, *ermB*, *ermF, imp13*, *mefE*, *mexB*, *qacF*, *qnrA*, *strB*, *sul1*, *sul2*, *sul3*, *tet*(A), *tet*(M), *tet*(W), *tet*(X), *vanA*) were quantified via real time polymerase chain reaction (PCR) from all 72 samples. Preliminary results showed that *blaSHV* (96% of sediment and 96% of water samples), *mexB* (92% of sediment and 96% of water samples), and *tet*(A) (92% of sediment and 91% of water samples) were the most common antibiotic resistance genes.

Final Report Summary
As per new information since the last progress reports, data for ARG showed that relative abundances in sediments were not associated with anthropogenic factors, but several of them (e.g., bla_{OXA}, *mexB*, and *sul2*) were inversely related to the organic content of sediments (Spearman's correlation = -0.38–-0.39, p = 0.04–0.03). Co-occurrence features were found among various ARG and *intI1* in sediments (Spearman's correlation ≥ 0.67, p < 0.05 for pairs of their relative abundances).

As summary for Activity 3, we were able to quantify the most frequent ARG in Minnesota water and sediment samples both at the macrospatial scale around the state and at specific sites influenced by wastewater treatment plant sources, highlighting the effect that these point sources have in the abundance of ARG. The information we have gathered on ARG can be useful to target specific ARG to be used as indicators of AMR in the natural environment.

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 wastewater, 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.

Activity Status as of January 1, 2019: Nothing to report.

Activity Status as of June 30, 2019: Nothing to report.

Third Update January 31, 2020

- On September 2019, the Environmental Protection Agency (EPA) office in Cincinnati hosted our postdoc Dr. Irene Bueno for two days. During those two days, Irene did a presentation about the project and met with different scientists from EPA to brainstorm methodologies to quantify and model antibiotic resistance in the environment, as well as future collaborations.

- An overview of the project and update of the 2019 field summer sampling was presented at the annual Minnesota One Health Antibiotic Stewardship Collaborative that took place on January 9th 2020 in Chaska, MN.

Fourth Update June 30, 2020: Nothing to report.

Fifth Update January 31, 2021:

- On February 10th, Dr. Irene Bueno will be presenting about the project to the University of Minnesota class 'GCC 1909 Introduction to Ecosystem Health: Challenges at the intersection of human, animal, and environmental health' (https://gcc.umn.edu/GCC1909).

Sixth Update June 30, 2021:

- On April 7th 2021, Dr. Huan He (Dr. William Arnold's lab) presented the preliminary results for antibiotic concentrations from the 2020 field season at the Annual conference of American Chemical Society (ACS). Her presentation was titled: 'Determination of antibiotic footprint in surface water environment of a metropolitan area'.
- On May 28th 2021, Dr. Irene Bueno presented the results from the soil vulnerability geospatial model, and Dr. Huan He presented preliminary results for antibiotic concentrations from the 2020 water samples, at the Minnesota One Health Antibiotic Stewardship Collaborative Annual Meeting. This meeting was virtual, had about 50 attendees from different disciplines, and the recording for it can be found here: https://www.youtube.com/watch?v=QFft7aN1Tmc.
- Dr. Irene Bueno has been invited to present our project 'Mapping Antibiotic Resistance in Minnesota to Help Protect Environmental, Animal, and Human Health' at the 2021 Virtual American Veterinary Medical Association (AVMA) Convention. Her presentation will be pre-recorded and presented online on 07/31/21 (https://s1.goeshow.com/avma/annual/2021/AVMA2021.cfm?session_key=BD74BACD-AF15-4197-246B-14A767B4A174&session_date=Saturday,%20Jul%2031,%202021).
- Dr. Amanda Beaudoin is leading the three working teams (animal, human, and environmental groups) from the Minnesota One Health Antibiotic Stewardship Collaborative (MOHASC) in creating educational materials for the general public to use at the Minnesota State Fair 2021. Our project team is contributing to this effort by providing some of the maps that were generated throughout our project.

Seventh Update January 31, 2022

- On 07/31/2021, Dr. Irene Bueno presented the results from this project at the American Veterinary Medical Association Conference (AVMA) virtually. The title of the talk was: "Minnesota Antibiotic Footprint".
- The State Fair 2021 did not have anybody in-person from this group due to COVID-19 limitations, so we could not present any educational materials. However, the intention is to develop educational materials that can be used in local libraries and perhaps in future state fairs.
- Publications:
    - We have one peer-reviewed publication showing 2018-2019 results: Bueno, I., Beaudoin, A., Arnold, W. A., Kim, T., Frankson, L. E., LaPara, T. M., ... & Singer, R. S. (2021). Quantifying and predicting antimicrobials and antimicrobial resistance genes in waterbodies through a holistic approach: a study in Minnesota, United States. Scientific Reports, 11(1), 1-15: https://www.nature.com/articles/s41598-021-98300-5.
    - We have another peer-reviewed publication "under review" which was submitted in December 2021 to the Science in the Total Environment Journal.

Final Report Summary

- New information since the last progress report:
  - Conference presentations:
    - Dr. Huan He presented results of the 2020 field season at the American Chemical Society (ACS) Spring 2022 National Meeting & Exposition (San Diego, CA) during March 20–24, 2022 (oral presentation title: Determination of the antibiotic and antibiotic resistance footprint in surface waters: Effects of anthropogenic activities); later, she also presented outcomes of this project at the 2022 Association of Environmental Engineering and Science Professors (AEESP) Conference (St. Louis, MO) during June 28–30, 2022 (poster title: Environmental and anthropogenic indicators for developing an antibiotic and antibiotic resistance footprint: A study of surface waters in the Twin Cities metropolitan area).
    - Undergraduates from the University of St. Thomas led by Sarah J. Ziemann presented results at the American Chemical Society Spring 2022 National Meeting. They presented a scientific poster titled "Fate and distribution of antibiotics in the natural environment: a field study in Minnesota and a scoping review". This poster included a mixture of results for this project and preliminary results for the newly funded ENRTF project.
    - Dr. Irene Bueno presented a summary of this project at the virtual Minnesota One Health Antibiotic Stewardship Collaborative Annual meeting (organized by the Minnesota Department of Health), celebrated on March 17th, 2022.
  - Teaching/Engagement: Dr. Irene Bueno taught at the Public Health Institute (University of Minnesota) on June 2nd, 2022, about antimicrobial resistance in the environment, including results from this project.
  - Publications: We published our work on soil vulnerability to antimicrobial contamination in a peer-reviewed journal: "Bueno, I., Rodríguez, A., Beaudoin, A., Arnold, W. A., Wammer, K. H., de la Torre, A., & Singer, R. S. (2022). Identifying the spatiotemporal vulnerability of soils to antimicrobial contamination through land application of animal manure in Minnesota, United States. *Science of the Total Environment*, 832, 155050. DOI: 10.1016/j.scitotenv.2022.155050". Also, a paper presenting the 2020 data on the spatial distribution of antibiotics and ARG is being finalized and will be submitted in the next few weeks.
- Overall summary of dissemination activities: This project has produced 2 peer-reviewed publications (Bueno, I., et al., 2021; and Bueno, I., et al., 2022) and there will be two others submitted soon summarizing the data for the 2020-2021 field seasons. This project has been presented at 9 (international and domestic) conferences both as poster and oral presentations, and during at least 2 teaching courses. Also, a graduate student used data from this project to conduct her Master's. One of the dissemination goals was to engage the general public at the state fair, but the COVID-19 pandemic halted that.

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	$195,000	Unrecovered F&A associated with research from U of MN (54%) during the three-year project period
Minnesota Department of Health	$18,728	$12,000	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 4 years, will begin on 7/1/18, and end on 6/30/22.**
- **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, 2022.**

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

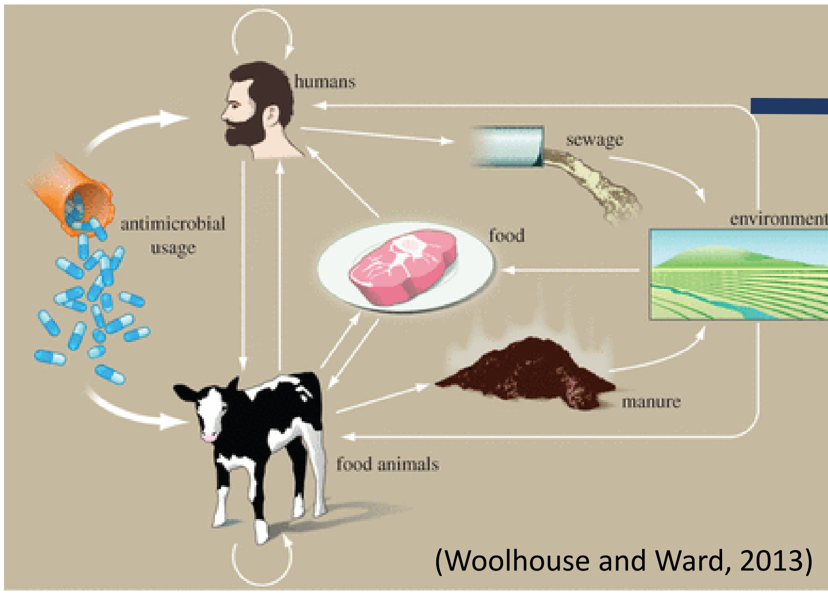
Attachment A:
Environment and Natural Resources Trust Fund
M.L. 2018 Budget Spreadsheet

Project Title: Mapping Antibiotic Resistance in Minnesota to Help Protect Environmental, Animal, and Human Health
Legal Citation: M.L. 2018, Chp. 214, Art. 4, Sec. 02, Subd. 04h
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: 4 years, June 30, 2022
Date of Report: August 15, 2022

ENVIRONMENT AND NATURAL RESOURCES TRUST FUND BUDGET	BUDGET August 15,	AMOUNT SPENT Activity 1	AMOUNT SPENT Activity 2	AMOUNT SPENT Activity 3	Total Spent	CURRENT BUDGET BALANCE
BUDGET ITEM						
Personnel (Wages and Benefits)	**$611,739**	**$268,545**	**$159,690**	**$182,389**	**$610,624**	**$1,115**
Randall Singer, project manager; $35,043 Activity 1 (including 33.5% fringe; 4% FTE years 1-3)		$35,043				
William Arnold, co-project manager; $23,867 Activity 2 (including 33.5% fringe; 4% FTE years 1-3)			$23,373			
Timothy LaPara, co-project manager; $26,370 Activity 3 (including 33.5% fringe; 4% FTE years 1-3)				$26,370		
Kristine Wammer, co-project manager; $5,219 Activity 2, (including 8% fringe; 5% FTE years 1-3)			$5,000			
Graduate student research assistant in Veterinary Medicine/UMN, data collection and analysis; $10,511 Activity 1 (61% salary, 39% fringe benefits; 50% FTE years 1&2, 25% FTE year 3)		$10,511				
Geospatial analyst in Veterinary Medicine/UMN, develop and validate the geospatial models; $222,991 Activity 1 (82% salary, 18% fringe benefits; 61% FTE years 1-3)		$222,991				
Graduate student research assistant in CEGE/UMN, sample collection, extraction, and analysis; $122,790 Activity 2 (58% salary, 42% fringe benefits; 50% FTE years 2&3)			$122,790			
Post-doctoral research associate in CEGE/UMN, sample collection, extraction, and analysis; $118,687 Activity 3 (82% salary, 18% fringe benefits; 100% FTE for years 2&3)				$118,687		
Undergraduates assist with sample collection and processing; $14,187 Activity 3 (paid hourly)				$13,785		
Undergraduates at UST, assist with sample collection, processing, and analysis; $8,527 Activity 2, $23,547 Activity 3 (paid hourly)			$8,527	$23,547		
Professional/Technical/Service Contracts	**$56,454**	**$0**	**$36,268**	**$20,186**	**$56,454**	**$0**
Analytical instrument time at UST;$0, Activity 3						
Analytical instrument time at UMN/Cancer Center; $36,268, Activity 2			$36,268			
Analytical instrument time at UMN/UMGC; $20,186, Activity 3				$20,186		
Equipment/Tools/Supplies	**$66,500**	**$0**	**$34,879**	**$30,631**	**$65,510**	**$990**
Supplies for sample collection, DNA extraction, and qPCR at UMN; $21,000, Activity 3				$20,381		
Supplies for sample collection, DNA extraction, and qPCR at UST; $10,500, Activity 3				$10,250		
Supplies for sample collection, extraction, and quantification of antibiotics at UMN; $35,000, Activity 2			$34,879			
Travel expenses in Minnesota	**$9,307**	**$1,216**	**$3,965**	**$2,441**	**$7,622**	**$1,685**
Travel to collect water and soil samples (vehicle rental, meals, and hotel); $5,460 Activity 2, $2,500 Activity 3			$3,900	$2,441		
Travel to attend in-state conferences to disseminate results; $1,247 Activity 1, $100 Activity 2,		$1,216	$65			
Other	**$6,000**	**$4,390**	**$1,000**	**$0**	**$5,390**	**$610**
Open access publication charges; $5,000 Activity 1,$1,000 Activity 2,		$4,390	$1,000			
COLUMN TOTAL	**$750,000**	**$274,151**	**$235,802**	**$235,647**	**$745,600**	**$4,400**

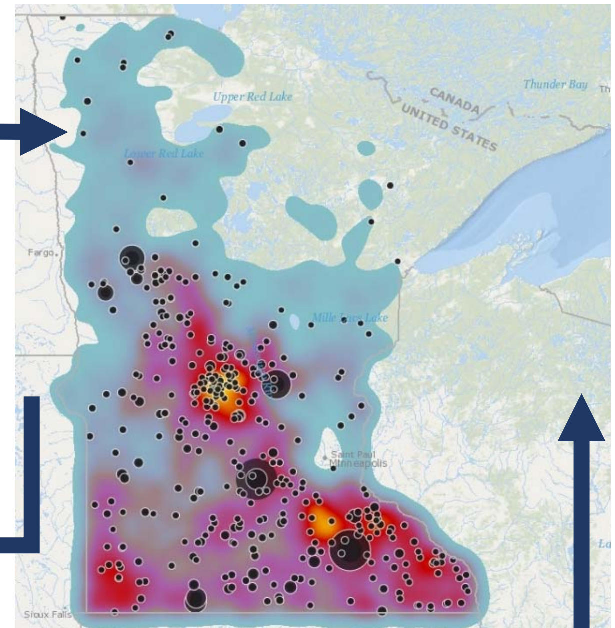
Mitigation of Antibiotic and Antibiotic Resistance Gene Pollution Requires Understanding the Sources of Contamination in Minnesota's Natural Environment

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