

1. Title of Proposal: Anthropogenic Influence on Tetracycline Resistance in a Rapidly Urbanizing Texas Stream

2. Focus Category: NON POINT POLLUTION ; SURFACE WATER ; WATER QUALITY

3. Keywords: antibiotic resistant genes, central Texas, gene transfer, water quality

4. Duration: March 1, 2010 through February 28, 2011

5. Federal Funds Requested. \$5,000

6. Non-Federal (matching) Funds Pledged: \$10,000 (Matching fund letter faxed to TWRI)

7. Principal Investigator (graduate student):

Bailey A. Sullivan
Third Year Doctorial Student
Texas A&M University
Department of Biological and Agricultural Engineering bas8844@tamu.edu
Office: (979) 862-8418
Cell: (785) 532-8040
2117 TAMU
College Station, TX 77843-2117

8. Co-Principal Investigator (faculty advisor).

Dr. R. Karthikeyan
Assistant Professor
Department of Biological and Agricultural Engineering
Texas A&M University
karthi@tamu.edu
Office: (979) 845-7951
2117 TAMU
College Station, TX 77843-2117

9. Congressional District(s) where project will occur: Texas Congressional District 17

10. Abstract:

The use of antibiotics in the medical field has led to the control of most bacterial diseases, unfortunately that ability has deteriorated due to the development of antibiotic resistance. Antibiotic resistant bacteria are ubiquitous in the environment and lead to increase in disease and death. Therefore it is important to minimize the spread of antibiotic resistance. The goal of my doctoral research is to study the occurrence,

prevalence, and fate of tetracycline resistant bacteria in a rapidly urbanizing Texas stream. The initial findings of my research showed that tetracycline resistant genes (TRGs) are present in a Central Texas watershed and there is a correlation between human activities, environmental media, and seasons on the occurrence and prevalence of TRGs.

In the proposed research, I will sequence resistant isolates and determine the dominant tetracycline resistant species and if the TRGs are carried on conjugable plasmids. Finally, I will determine the effects of nutrients, tetracycline, and heavy metals on the kinetics of tetracycline resistant genes in water. Results from this research will help us better understand the fate of TRGs in the watershed and provide insights into possible management practices to minimize the spread of TRGs in surface waters.

11. Statement of Critical Regional Water Problems

There are several studies conducted elsewhere in the nation documenting the presence of antibiotic resistant bacteria in the environment (Aminov *et al.*, 2001; Chopra *et al.*, 2001; Cohen, 1992; Davison, 1999; McAurthur *et al.*, 2000; Pei *et al.*, 2006; Purden *et al.*, 2006). Carters Creek watershed is a sub-watershed of Navasota River Basin in East Central Texas. This watershed is located in Brazos County and covers about 57 square miles. The 17 mile long Carters Creek (Segment #1209C) passes through land use that is heavily urbanized in the upper reach of the watershed where it originates in Bryan/College Station, and becomes more rural in the lower reach. This Creek is fed by two wastewater treatment facilities, urban runoff, and agricultural runoff. Carters Creek is currently on 303(d) list for bacterial impairment. According to 2006 303(d) list, there are 197 streams in Texas that are impaired due to bacteria. The bacterial impairment is based on exceeding the regulatory standard of either geometric mean of 126 CFU *E. coli*/100 mL or single sample maximum of 396 CFU *E. coli*/100 mL. Logically, these bacterial-impaired streams can be considered as potential sources of antibiotic resistant bacteria contamination as well. If these impaired streams turned out to be carrying resistant bacteria, the issue becomes very critical and warrant immediate action. My two-year research showed that three tetracycline resistant genes are present in sediments and water collected from Carters Creek watershed.

12. Nature, Scope, and Objectives of the Research.

The overall objective of my doctoral research is to determine the anthropogenic influences on the occurrence of tetracycline resistance genes and to aid in the development of best management practices to minimize the spread of tetracycline resistance genes in the environment. Specific objectives are as follows.

Objective 1 (completed): Determine the effects of land use (Agricultural, Urban, and Pristine), seasonality (fall/winter and spring/summer) and environmental media (sediment and water) on the occurrence of three tetracycline resistance genes (*tet(O)*, *tet(W)* and *tet(Q)*). Carters Creek will be monitored to test the following hypotheses:

- a) Antibiotic resistance genes (ARGs) will be significantly higher in areas that are affected by human activities such as agriculture and urbanization as compared to pristine conditions.
- b) ARGs will be significantly higher during the fall/winter season than the spring/summer season.
- c) ARGs will be significantly higher when extracted from sediment than from water.

Objective 2 (proposed): Determine the dominant species of tetracycline resistant bacteria and if tetracycline resistant isolates contain conjugable ARGs. Conjugation experiments will be performed with 100 tetracycline resistant isolates to test the following hypotheses:

- a) Majority of the tetracycline resistant isolates will be the same species.

- b) Majority of the tetracycline resistant isolates will contain conjugable ARGs.

Objective 3 (proposed): Determine the effects of nutrients (nitrogen, phosphorous, and organic carbon), tetracycline, and heavy metal concentration (zinc, mercury, and cadmium) on the kinetics and concentration of a tetracycline resistant gene. Microcosm study will be preformed to test the following hypotheses:

- a) Increased nutrient concentrations will not significantly affect the copy number ratio of ARGs.
- b) Increased levels of tetracycline will significantly increase the copy number ratio of ARGs.
- c) Increased levels of heavy metals will significantly increase the copy number ratio of ARGs.

13. Results Expected from this Project

Objective 1: I have collected 10 sediments and water samples from Carters Creek over the period of two-years from five different locations. I have screened the samples by molecular methods such as Polymerase Chain Reaction (PCR) and regular culture-based methods. The initial findings showed that three tetracycline resistant genes are present in a watershed and there is a reasonable correlation between human activities, land use, environmental media, and seasons on the occurrence and prevalence of tetracycline resistant genes. I have isolated heterotrophic bacteria resistant to tetracycline by culture based methods.

Objective 2: It is expected that the dominant species between sites will be similar, indicating that the resistance came from a common ancestor. There could be slight discrepancies between isolates collected from soil and those from water due to the different microenvironments. However it is hypothesized that the resistant species within the watershed stemmed from similar origins and environmental pressures. It is expected that a majority of the isolates will contain their tetracycline resistance genes on conjugable plasmids because most of the resistance genes are highly mobile (Kruse *et al.*, 1994).

Objective 3: It is expected that tetracycline and heavy metal treatments will increase the growth rate of tetracycline resistant organisms when compared to nutrient and control treatments. This would indicate that the treatments increase the number of tet(W) genes in the microcosm. It is also expected that there will be no significant differences between the growth curves of the resistant organisms of the metal treatments and the tetracycline treatments, since most ARGs are carried on the same plasmids as metal resistance (Baker-Austin *et al.*, 2006). It is predicted that the growth curves of the resistant organisms between tetracycline and nutrient treatments will be significantly different, since the nutrient treatment exerts no pressure on the bacterial cell to maintain the tetracycline resistance. Finally it is expected that the resistance growth curves between mixed and isolate treatments will be significantly different due to competition in the natural treatment.

References

- Aminov, R.I.; Garrigues-JeanJean, N.; Mackie, R.I. Molecular Ecology of Tetracycline Resistance: Development and Validation of Primers for Detection of Tetracycline Resistance Genes Encoding Ribosomal Protection Proteins. *Appl Environ Micro.* 2001, 67, 22-23.
- Baker-Austin, C.; Wright, M.S.; Stepanauskas, R.; McArthur, J.V. Co-selection of antibiotic and metal resistance. *Micro.* 2006, 14, 176-182. *App & Enviro Micro.* 2001, 67, 22-32.
- Chopra, I.; Roberts, M. Tetracycline Antibiotics: Mode of Action, Applications, Molecular Biology, and Epidemiology of Bacterial Resistance. *Micro & Molec Bio Review.* 2001, 65, 232-260.
- Cohen, M.L. Epidemiology of Drug Resistance: Implications for a Post-Antimicrobial Era. *Science.* 1992, 257, 1050-1055. Davison, J. REVIEW Genetic Exchange Between Bacteria and the Environment. *Plasmid* 1999, 42, 73-91.
- Davison, J. Review Genetic Exchange between Bacteria in the Environment. *Plasmid.* 1999, 42, 73-91.
- Iwane, T.; Urase, T.; Yamamoto, K. Possible impact of treated wastewater discharge on incidence of antibiotic resistant bacteria in river water. *Wat. Sci. Tech.* 2001, 43, 91-99.
- Kruse, H.; Sorum, H. Transfer of Multiple Drug Resistance Plasmids between Bacteria of Diverse Origins in Natural Microenvironments. *Apl & Environ Micro.* 1994. 4015-4021.
- McArthur, J. V.; Tuckfield, R. C. Spatial Patterns in Antibiotic Resistance among Stream Bacteria: Effects of Industrial Pollution. *Appl. Environ. Microbiol.* 2000, 66, 3722-3726.
- Pei, R.; Kim, S.; Carlson, K. H.; Pruden, A. Effect of River Landscape on the sediment concentrations of antibiotics and corresponding antibiotic resistance genes (ARG). *Wat. Res.* 2006, 40, 2427-2435.
- Pruden, A.; Pei, R.; Storteboom, H.; Carlson, K.H. Antibiotic Resistance Genes as Emerging Contaminants: Studies in Northern Colorado. *Environ. Sci. Technol.* 2006, 40, 7445-7450.