Antibiotic resistance is an ancient phenomenon born of microbial interactions and furthered by mutations and horizontal gene transfer (4). The rise of antibiotic resistance has created a major public health crisis as humans rely heavily on antibiotics to control infectious diseases, which are now becoming increasingly resistant to extant treatments. In past studies, it has been discovered that homologous multi-drug efflux pumps are conserved across species of microorganisms, but small differences can result in different determinants of resistance; this conservation also suggests that the multi-drug resistant efflux pump is an ancient mechanism that has been evolutionarily preserved in microorganisms (2). I will contribute to our understanding of the fundamental processes underlying antibiotic resistance, thus allowing us to design more effective drugs and preserve our antibiotic drug arsenal. Most research conducted on this topic has characterized the MexAB-OprM efflux pump in *Pseudomonas aeruginosa*, a model organism and an opportunistic pathogen mainly affecting cystic fibrosis patients. It has been found that this efflux pump is a major determinant of resistance to antibiotics and biocides in *P. aeruginosa* (3). However, little research has been conducted on this homologous system in other strains. In this project, I will assess the genetic factors involved in antibiotic resistance in different strains of the bacteria *Pseudomonas.* By explaining how this system works in *P. fulva* and *P. stutzeri*, I will further our understanding of antibiotic resistance in general. I would like to find out how differences in the amino acid sequences of the MexAB-OprM operon result in different levels of susceptibility in the different strains.

The two strains I plan to study are *P. fulva* and *P. stutzeri* as it has already been established via previous experiments that these non-model strains are resistant to the antibiotic of interest: triclosan (TCS). I hypothesize that resistance in these strains, like in *P. aeruginosa*, is determined by their MexAB-OprM homolog and that interruptions to the genes encoding that efflux pump will result in a loss of resistance. To test that hypothesis, I will conduct transposon mutagenesis, a process that involves transforming a strain of *Pseudomonas* with a plasmid containing a transposon. Specifically, the plasmid I am using is the SM10 plasmid from an *Escherichia coli* cell containing the Hinmar1 transposon and a gentamycin resistance gene (5). The transposon will insert at specific sites within the *Pseudomonas* genome. If the transposon inserts into a gene responsible for antibiotic resistance, this phenotype will be knocked out and the mutant colony will be susceptible to TCS. Colonies that are successfully transformed in this way will then be sequenced to see which genes are necessary for antibiotic resistance in different strains of *Pseudomonas*.

Past research has revealed that mutations in the efflux pump, encoded by the *mexA, mexB,* and *oprM* genes, are correlated with reduced resistance to certain biocides in *P. azelaica* HBP1 (1). Therefore, I will look at *Pseudomonas* mutants that no longer exhibit TCS resistance to further characterize the mutations and determine if this efflux pump regulates antibiotic susceptibility. I will also compare sequences of this operon across different strains to determine if differences in the efflux pump genes correlate to different levels of resistance as determined by the minimum inhibitory concentration (MIC) of TCS. I will align the sequences of these homologous genes and evaluate their percent similarities using the EMBOSS package (6). I will then determine if sequence similarity correlates with the MIC using linear regression. I also plan to use principle component analysis to see how similarities in the sequences of the pumps correlate with different MICs between mutant and wildtype colonies. Finally, I will use the sequencing data to evaluate how changes in key amino acid residues within the pump sequence affect the MIC by designating residues as specific variables and performing a T-test to check for statistically significant data points.

I will create 4,000 mutants of an isolate of *P. fulva* and 4,000 mutants of an isolate of *P. stutzeri* because the *Pseudomonas* genome contains approximately 4,000 genes. Therefore, by

creating 4,000 mutants I will likely have interrupted all non-essential genes at least once, giving me ample opportunity to identify genes involved in susceptibility to TCS. To create the mutants, I first culture the wild type overnight in tryptic soy broth (TSB), and then conjugate with an isolate of *E. coli* which contains the SM10 plasmid. To select for *Pseudomonas* colonies that have successfully undergone conjugation, I then dilute the conjugated colonies in phosphate buffer solution (PBS) and grow for 48 hours on a selective minimal media agar plate with gentamycin plus factors such as sodium succinate and M63 salts that inhibit *E. coli* growth and promote *Pseudomonas* growth. After selecting for the conjugated colonies, I will grow them overnight in TSB, and then transfer them to a tryptic soy agar + TCS plate. The wild type *P. fulva* was previously able to grow in the presence of TCS at 128.0 mg/L. If the mutants are no longer able to grow in the presence of TCS at this concentration, the transposon likely knocked out a gene involved in antibiotic resistance. I will use Sanger sequencing to determine whether the transposon inserted into the *mexAB-oprM* operon. Figure 1, inserted below in appendix 2, shows the insertion map of the mutant colony that exhibited decreased resistance after mutagenesis. This figure illustrates that the transposon was inserted into the *mexA* region of the genome, knocking out the resistance phenotype.

So far, I have created 1,500 mutants of *P. fulva* via conjugation and transposon mutagenesis. Out of the 1,500 mutants thus far created, one colony was unable to grow in the presence of TCS. Based on the sequencing results, the transposon was inserted into the *mexAB-oprM* operon. For *P. fulva*, the MIC of the mutant decreased to 8.0 mg/L, showing how antibiotic resistance decreased when the transposon was inserted into the MexAB operon. In addition to repeating this experiment with *P. stutzeri*, I would like to be able to continue this work with *P. fulva* to further assess the factors of antibiotic resistance and to see which other genes influence levels of resistance.

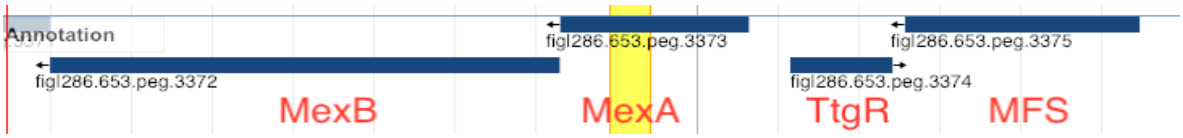
To prepare for this lab work, I have completed the biology 220 laboratory, general chemistry 121 laboratory, organic chemistry 230 laboratory sequences as well as courses in genetics, cell biology, physiology, animal physiology, biochemistry, organic chemistry, general chemistry and international public health. Finally, I have been enrolled in civil and environmental engineering 399 with Dr. Hartmann for 2 quarters and am currently enrolled in advanced cell biology and microbiology. Each of these courses has helped me learn the skills necessary to complete this research project. In the future, I would like to continue to study microbiology to better understand the mechanisms behind antibiotic resistance and the dangers of infectious pathogens. As both of these topics are an increasing threat to public health and are especially threatening to vulnerable populations such as hospitalized persons and those who are immunocompromised, I intend to pursue a degree in microbiology to further understand the pathogens posing these threats. I hope to use this information to discover new ways to combat antibiotic resistance and other virulent pathogens threatening our public and global health.

Appendix 1: References

1. Kamila, et al. “Characterization of a MexAB-OprM Efflux System Necessary for Productive Metabolism of Pseudomonas Azelaica HBP1 on 2-Hydroxybiphenyl.” *Frontiers*, Frontiers, 28 June 2013, www.frontiersin.org/articles/10.3389/fmicb.2013.00203/full.

2. Blanco, Paula, et al. “Bacterial Multidrug Efflux Pumps: Much More Than Antibiotic Resistance Determinants.” *MDPI*, Multidisciplinary Digital Publishing Institute, 16 Feb. 2016, www.mdpi.com/2076-2607/4/1/14.
3. Lambert, P A. “Mechanisms of Antibiotic Resistance in Pseudomonas Aeruginosa.” *National Center for Biotechnology Information*, Journal Of The Royal Society Of Medicine, 2002, www.ncbi.nlm.nih.gov/pmc/articles/PMC1308633/pdf/12216271.pdf.
4. Martínez, José L. “Antibiotics and Antibiotic Resistance Genes in Natural Environments.” Science, American Association for the Advancement of Science, 18 July 2008, science.sciencemag.org/content/321/5887/365?casa_token=pH3-LdlrIDkAAAAA%3A-EWqiJwAxnFZeTaOpWxF3V3Ysz3VQCeUbQyHSpYroQhJpz0JBzV38Pcy6XWuiRENG85PdfhoRk2Irw.
5. Strand, Trine Aakvik, et al. “A New and Improved Host-Independent Plasmid System for RK2-Based Conjugal Transfer.” PLOS ONE, Public Library of Science, journals.plos.org/plosone/article?id=10.1371%2Fjournal.pone.0090372.
6. Rice, P, et al. “EMBOSS: the European Molecular Biology Open Software Suite.” Current Neurology and Neuroscience Reports., U.S. National Library of Medicine, June 2000, www.ncbi.nlm.nih.gov/pubmed/10827456.

Appendix 2: Figure 1



Appendix 3: Itemized Budget

1. Sanger sequencing
2. Reagents and media
    a. Bacto agar
    b. Sodium succinate
    c. Magnesium chloride
    d. Triclosan
    e. Gentamycin
    f. Ferrous ammonium sulfate
    g. L-glutamic acid
    h. Dibasic potassium phosphate
    i. Potassium dihydrogen phosphate
    j. Ammonium sulfate