

Metagenomic Analysis and Functional Screening of Antibiotic Resistance Genes in the Santa Cruz River

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Antibiotic resistance is a growing global concern as it is associated with at least 2.8 million infections and 35,000 deaths in the United States alone every year. Globally, it is estimated to result in over 1.2 million deaths annually. Antibiotic resistant bacteria (ARB) are commonly present in sewage and can be spread through sewage leaks. Since at least 2017, the Santa Cruz River has experienced frequent sewage leaks from the International Outfall Interceptor (IOI) that serves Nogales on both sides of the border, resulting in millions of gallons of raw sewage being dumped into the river for the last few years. To assess the impact these sewage leaks had on microbial communities, pathogens, and levels of antibiotic resistant genes (ARGs) in this critical water source, sediment samples were taken in triplicate from near the leaks (Nogales) and two locations away from the contamination point (Tubac and Marana) at four different time points between October 2019 and October 2020. DNA was extracted from each sample (n=108), sequenced, and used for shotgun metagenomic analysis with the MetaWRAP pipeline and additional programs and/or databases including Kraken2 (taxonomy), Abricate and DeepARG (ARGs), and PathogenFinder and Virulence Factor Database (pathogens). Confirmation of functional capabilities of identified ARGs in the various samples was conducted by cloning sample DNA into pSMART-BAC, transforming *Escherichia coli* DH5 α and screening transformants against different concentrations of the target antibiotic. Our results show that there is a large number of antibiotic resistance genes to a variety of classes present in all the sediment samples. The most common antibiotic classes that were identified bioinformatically were tetracycline, beta-lactams, and aminoglycosides. The goal of this project is to understand the impact numerous sewage leaks have on introducing ARGs into critical water system, and how those are then dispersed throughout the water system over time.