

## Antibiotic Resistance Genes (ARG) Studies in the Poudre River and Northern Colorado

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**Abstract.** The spread of antibiotic resistance genes (ARG) has been associated with the widespread use of antibiotics in livestock as well as humans. Since ARG are being recognized as emerging contaminants, there is a need to better understand their sources, pathways, and ultimate fates in the environment. The purpose of this study was to quantify the spatial and temporal variation of ARG in the mixed-landscape Cache La Poudre River sediments, which have previously been studied and shown to have high concentrations of antibiotics correlating with urban and agricultural activity. Additionally, irrigation ditches and lagoons were monitored within the watershed as potential sources of ARG. In order to detect and quantify ARG in various environmental compartments, molecular biological methods were developed and applied. Polymerase chain reaction (PCR) detection assays were conducted for seven sulfonamide resistance gene families using newly designed primers and eight tetracycline resistance gene families using previously published primers. *Sul(I)*, *sul(II)*, *tet(W)*, and *tet(O)* gene families were further quantified by real-time quantitative polymerase chain reaction (Q-PCR). Resistance to four classes of antibiotics (tetracyclines, sulfonamides, ionophores, and macrolides) was also investigated using a culture-based approach on antibiotic media. ARG were found to be present in river sediments, and their concentrations correlated to human and agricultural activity. The quantities of ARG normalized to the 16S rRNA gene copy number were statistically different between the sampling sites with different levels of human and agricultural input, and the pristine site being the lowest in all sampling events. These results suggest the need for increased involvement of environmental engineers to help understand the pathways of ARG in the environment and to develop treatment and mitigation strategies for minimizing the spread of ARG in the environment.

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