



## USDA-CSREES 2006 National Water Quality Conference

### Antibiotic Resistance Genes (ARG) in the Watershed

We are investigating antibiotic resistance genes (ARG) as emerging contaminants in the watershed environment. The spread of ARG is a growing concern considering the increasing microbial resistance to antibiotics that is being observed world-wide. The purpose of this study was to quantify the occurrence of antibiotic resistance in the mixed-landscape Cache La Poudre River watershed, which has previously been studied and shown to have high concentrations of antibiotics correlating with urban and agricultural activity. In order to detect and quantify ARG in the environment, molecular biological methods were developed and applied to the environmental samples. 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 Cache La Poudre River sediments, and overall concentrations correlated with 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, with the pristine site being the lowest in all sampling events. Overall, these findings were consistent with the land use types and extent of the inputs. These results suggest the need for research to understand the pathways of ARG in the watershed environment and development of treatment and mitigation strategies for minimizing the spread of ARG.

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