



# Indirect selection for antibiotic resistant bacteria in heavy metal contaminated streams

## Overview

### Antibiotic resistant bacteria are routinely isolated from the environment

-Typical mechanisms for maintaining antibiotic resistance (AR): releases of antibiotics or resistant bacteria from animal and sewage operations

-Our studies at the Savannah River Site (SRS) indicate that heavy metal contamination is an additional mechanism for maintaining AR in the environment

-This mechanism likely operates through an association between metal tolerance (MT) and AR genes that are linked on mobile genetic elements, thus when one trait is directly selected for, the other trait is then indirectly selected.

**Research question:** What factors influence the transfer and transport of antibiotic resistance in heavy metal contaminated streams?

We predict that factors that alter the degree of selective pressure (eg, metal concentration, DOC, pH) and factors that influence bacterial transport (eg, flow rate, intensity of bacterial habitat disturbance) will influence the transfer and transport of AR genes

### Relevant spatial scales for studying AR in the environment:

- Molecular scale: gene *transfer* between bacterial genomes
- Watershed scale: gene *transport* through the stream

## Approach

### Study System: Savannah River Site

-Contaminated sites: Coal fired power plant and its associated ash settling basins are a point source of metal contamination in Beaver Dam Creek

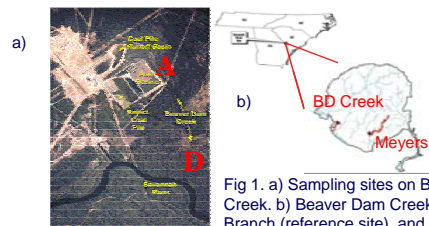


Fig 1. a) Sampling sites on Beaver Dam Creek. b) Beaver Dam Creek, Meyers Branch (reference site), and the Savannah River Site.

### Observational Studies:

1. Compare AR and MT frequencies between contaminated and reference sites, between microhabitats within a site, and over time
2. Measure abiotic factors that may correlate with observed AR and MT frequencies
3. Characterize the microbial community between sites & microhabitats using molecular techniques
  - Predict that sites and microhabitats with the highest AR and MT frequencies will be important sites for AR transfer and transport

### Experimental Studies:

1. Manipulate biotic and abiotic factors to directly test their affect on AR and MT transfer and transport rates using a series of microcosm studies
2. Use gfp-labeled bacteria to track transfer and transport

## Transplant study

### Objective

1. Identify bacterial microhabitats within the stream system that have high frequencies of AB and and MT
  - Water column
  - Sediment
  - Biofilm
  - Invertebrate digestive tract: *Corbicula fluminea*, a freshwater clam
2. Investigate temporal patterns in AB and MT

### Methods

Transplanted biofilm and *Corbicula* from Meyers (reference) to the two sites on Beaver Dam and back-transplanted to Meyers, and then measured AR and MT frequency over three months using culture-independent techniques

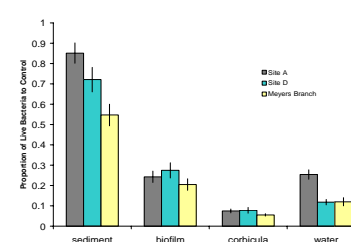


Fig 2. AR and MT for each of the sites and microhabitats averaged over 3 months of sampling ( $\pm 1$  s.e., n=60).

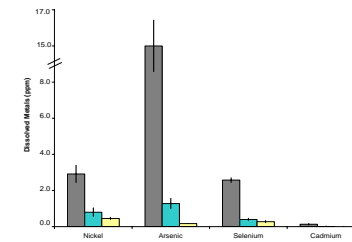


Fig 3. Dissolved metal concentrations averaged over 3 months of sampling ( $\pm 1$  s.e., n=3).

### Results and Discussion

1. Sediment had highest AR and MT: likely to be important source of AR and MT genes
2. Site A had the highest metal concentration and the highest AR and MT: supports hypothesis that metal contamination indirectly selects for antibiotic resistance

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