Identification of Isolated Antibiotic Resistance Genes within the Blue Marsh Watershed

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ABSTRACT

Bacteria can be found everywhere. Most bacteria are benign or beneficial, however, some are pathogenic. The threat begins when a pathogen acquires antibiotic resistance. Antibiotic resistance occurs when bacteria develop a mechanism to render antibiotics ineffective. When pathogenic antibiotic-resistant bacteria are introduced to an area of high-water usage it poses a public health risk. Watersheds like Blue Marsh are potential reservoirs for antibiotic-resistant pathogens. The primary objective of this project is to determine the prevalence of antibiotic-resistant genes in the Blue Marsh reservoir. Three areas within the reservoir serve as sites for analysis of antibiotic-resistant genes. All sites are exposed to agricultural, industrial, or recreational uses making them potential areas where high levels of antibiotic-resistant pathogens may be found. We analyzed the microbial population within the Blue Marsh watershed. For the analysis, we isolated genomic DNA from sediment samples collected over a period of one year. Isolated genomic DNA was analyzed for antibiotic-resistant genes using PCR techniques. Tetracycline resistant genes were found in several samples. The presence of the antibiotic resistance genes found in the Blue Marsh watershed sediment samples suggests that potential pathogens may be present due to the human action in and around this waterway.

SAMPLE SITES

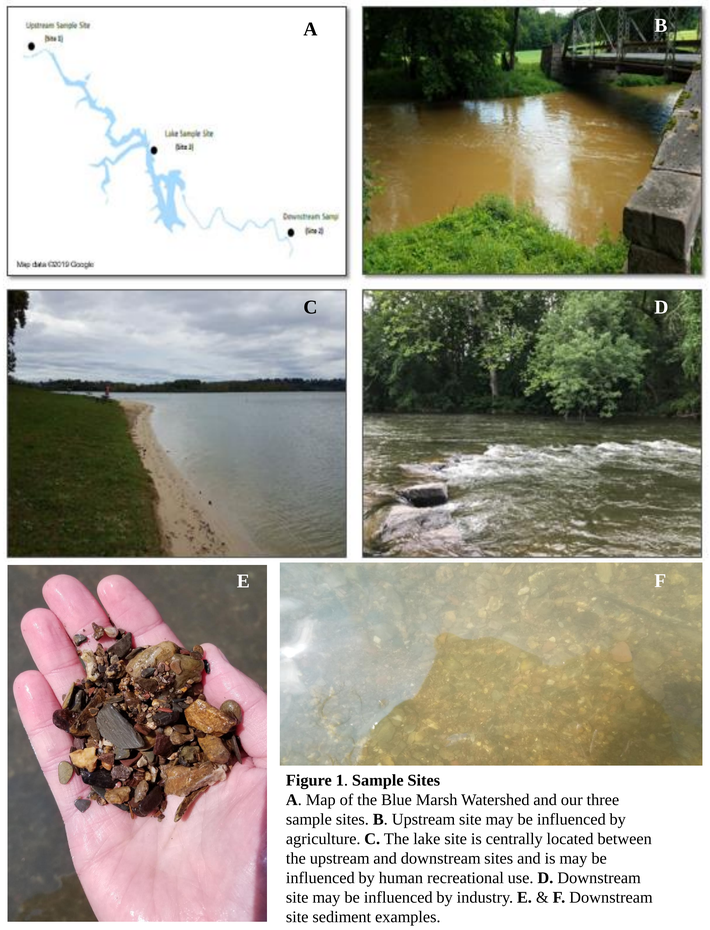

Figure 1. **Sample Sites**
A. Map of the Blue Marsh Watershed and our three sample sites. **B**. Upstream site may be influenced by agriculture. **C.** The lake site is centrally located between the upstream and downstream sites and is may be influenced by human recreational use. **D.** Downstream site may be influenced by industry. **E.** & **F.** Downstream site sediment examples.

RESULTS

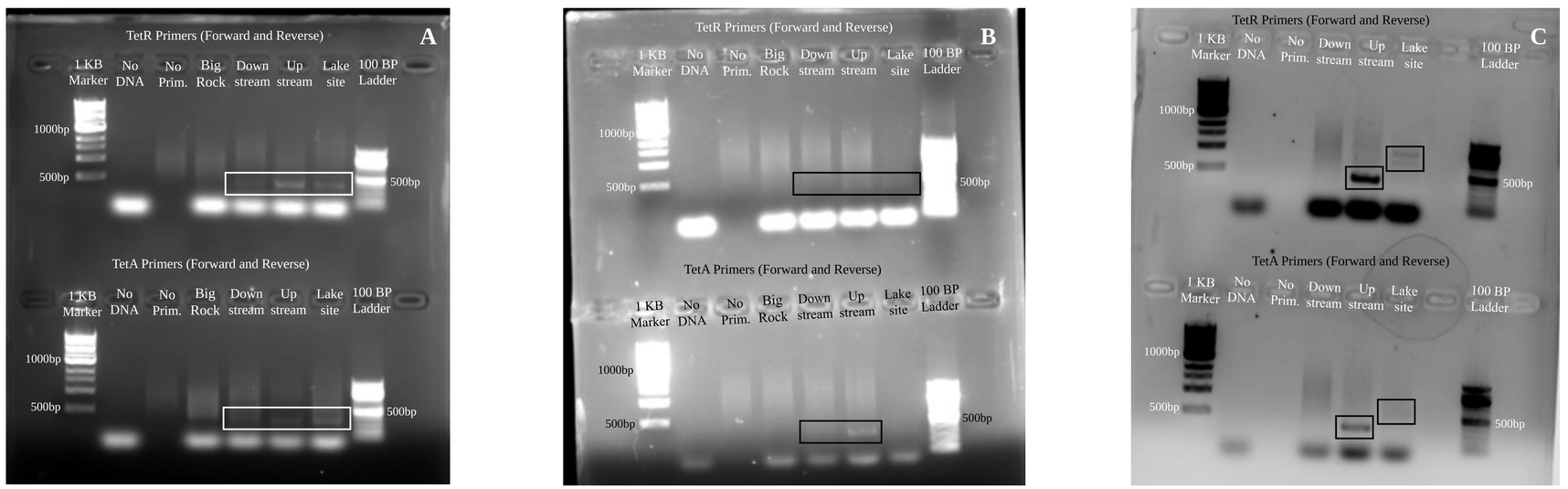

Figure 2. **PCR analysis of Sediment from Sample Sites Using Tetracycline Resistant Gene Specific Primers**
A. May Soil Sediments. **B**. August Soil Sediments. **C.** September Soil Sediments.

MATERIALS AND METHODS

- Sediment samples were collected each month from all three sites of Blue Marsh watershed along with another site (labeled as Big Rock in Figure 2). This site served as our non-point source sample (negative control).
- Total genomic DNA was isolated using PowerSoil DNA Extraction Kit, according to manufactures protocol (Qiagen).
- PCR amplification was performed on genomic DNA using tetracycline resistance gene primers TetA and TetR (forward and reverse primer pairs). Primers were designed using GenBank sequence and Primer3 software analysis.
- PCR products were analyzed using agarose gel electrophoresis (1% agarose, 100V, 25minutes) and visualized using Odyssey imaging system, LiCor.

FUTURE DIRECTIONS

- Test environmental samples for additional common antibiotic resistance genes using PCR analysis and PCR primers specific for the following antibiotic resistance genes.
  - Ampicillin
  - Cirpoflaxocin
- Sequence the PCR products which represent the antibiotic resistance gene to confirm our conclusion that these genes are present in the environment.

RESULTS AND CONCLUSION

- The bands detected (within the rectangles in Figure 2) in lanes from genomic DNA isolated sediment at specified sample sites at specific times indicate the presence of tetracycline resistance genes in the environment.
- Figure 2A shows the presence of tetracycline resistance genes at all three sites (Upstream, Downstream and Lake Site) as identified with both primer sets for tetracycline resistance genes (A and R) during the month of May.
- Figure 2B shows the presence of tetracycline resistance gene tet R at all three sites (Upstream, Downstream and Lake Site) and resistance gene tetA at two sites (Upstream and Downstream) during the month of August.
- Figure 2C shows the presence of tetracycline resistance gene tet R and tet A at two sites (Upstream and Downstream) during the month of September.

Our results suggest that antibiotic resistance genes are commonplace in our environmental samples collected within the Blue Marsh Watershed and that these genes may raise public health concerns.

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