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The effect of combined sewer overflow (CSO) on the abundance of antibiotic resistant bacteria in the James River

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Introduction

- Antibiotics have been used to treat bacterial infections worldwide since their discovery in the early 20th century and are vital to human health. Unfortunately, the heavy use of antibiotics has led to the increased natural selection of antibiotic resistant bacteria[1].
- In urban rivers, the spread of resistance is through the direct acquisition of resistance genes by either cell-to-cell contact or DNA uptake via a process called horizontal gene transfer (HGT)[2]. HGT, resistance genes, and resistant bacteria are in greater abundance in wastewater systems, and are released into the environment in wastewater plant effluent[2,3].
- One problematic method of wastewater treatment, used in over 750 cities in the US, is the Combined Sewer System (CSS)[4]. This collects the water from both rainfall and sewage for treatment at a single facility. Occasionally when it rains, the treatment plant exceeds capacity and the combined untreated effluent enters the river in what is called a CSO (Combined Sewer Overflow) event.
- Some studies have found that antibiotic resistance genes can be more abundant in river water affected by wastewater treatment effluent[5,6] and correlated with CSO events[7].

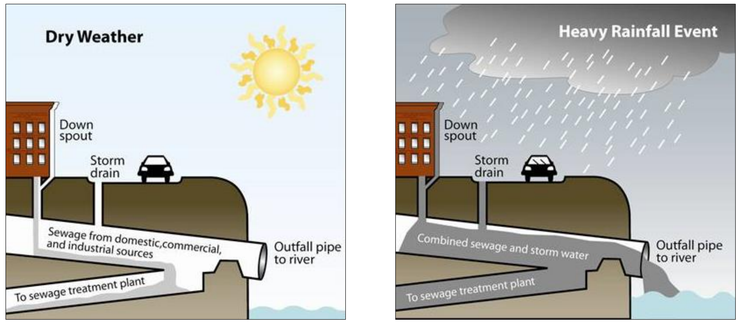

Figure 1. Overview of Combined Sewer System[4].

Objectives

1. Determine if CSO events are correlated with an increased abundance of antibiotic resistance genes compared to base flow conditions.
2. Isolate and identify specific species of bacteria that are antibiotic resistant and determine their level of multi-drug resistance.
3. Quantify the abundance of genes associated with antibiotic resistance in the river microbial community and determine the spatial constraints on their abundance.

Study Site

In Richmond, the James River is the major source of drinking water and widely used for recreation. Upstream, the watershed is primarily forested and agricultural land, but is also impacted by two major cities, over 150 industrial sites, and over 90 additional discharge sources[8,9]. The first sampling site is upstream of the city, near the Huguenot Bridge. The second site is at the largest CSO outfall point in Richmond, CSO-06 (Shockoe).

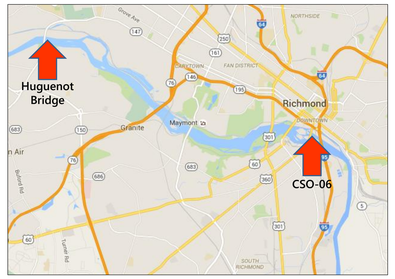

Figure 2. Sampling sites. Image created using Google Maps.

Colony Counts

- The CSO site was sampled during base flow conditions ("nonevent", 28 June 2016) and during a CSO event (5 July 2016). The Huguenot Bridge (HUG) site was only sampled during the nonevent.
- Heterotrophic plate counts were performed using R2A agar (for total bacterial abundance) as well as either ampicillin-, streptomycin-, or tetracycline-amended R2A agar.
- Antibiotic resistant bacteria were more prevalent during the CSO event than during the nonevent. *E. coli* counts during a CSO event were above the EPA guidelines for primary contact counts.

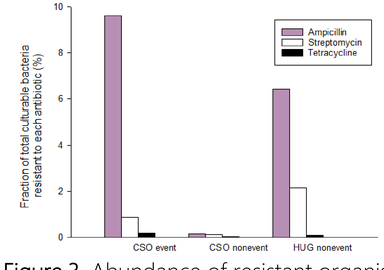

Figure 3. Abundance of resistant organisms.

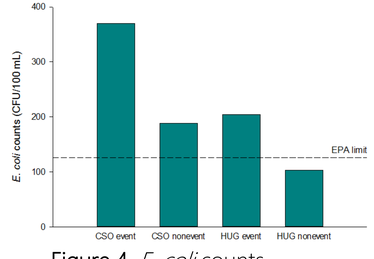

Figure 4. *E. coli* counts.

Antibiotic Resistance Genes

- For the next step of this project we plan to monitor the abundance and distribution of antibiotic resistance genes using quantitative PCR.
- The resistance genes of interest are spread on mobile genetic elements and may come from anthropogenic inputs.
- The genes of interest confer resistance to β-lactams (bla_{TEM}), erythromycin (*ermB*), quinolones (*qnrA*), and tetracycline (*tetA*).

Multi-Drug Resistance (MDR)

- Isolates were then screened for multi-drug resistance (MDR) using ampicillin (A), streptomycin (S), and tetracycline (T).
- MDR was greatest at the CSO site during an event: 70% of isolates showed MDR and 49% were resistant to all three antibiotics.
- Additional MDR tests are being conducted with susceptibility disk tests using more clinically relevant antibiotics: piperacillin, ciprofloxacin, cefepime, cefotaxime, Bactrim, and Augmentin.
- Sanger sequencing of the *16S rRNA* gene will be used to identify these isolates.

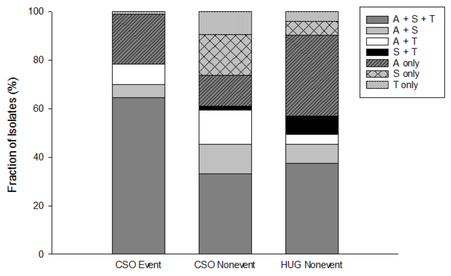

Figure 5. Fraction of isolates that were resistant to a combination of or only: ampicillin (A), streptomycin (S), or tetracycline.

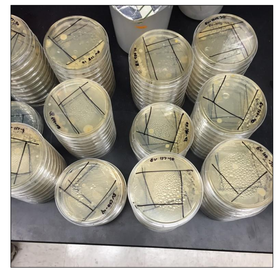

Figure 6. Bacterial streaks for isolation during MDR testing.

Acknowledgements

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