Validation and application of qPCR-MST of fecal contamination in the Mohawk River Watershed

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Abstract

Fecal indicator bacteria (FIB) are often used to determine whether a waterbody can be safely used for recreation. However, FIB are not source specific and provide minimal information on the source of contamination. Therefore, the combination of FIB and new techniques such as microbial source tracking (MST) have the potential to refine information for remedial efforts. In the summer of 2019, water samples from the western portion of the Mohawk river watershed were evaluated for FIB and the occurrence of two source specific markers: human and bovine. In addition to providing proof of concept for MST in this specific location, the study showed wide spread detection of the human marker HF183 in over 50% of the samples. The marker was the most prominent within the city of Utica, where detection of HF183 occurred in 69-77% of the samples and is likely due to the impact of the network of combined sewer overflows (CSOs). Overall, there was strong co-occurrence between elevated FIB concentrations and source markers in the region and vice versa (low FIB concentrations and no marker detection). However, 35% of samples with elevated FIB concentrations had no detection of the human or bovine markers suggesting the potential of a significant contribution from a source not evaluated in this study.

Three Summary Points of Interest

- 148 samples were collected and analyzed for the presences / absence of human and bovine markers
- The assay HF183 (human) was detected in 52% of the samples while COWM3 was detected in 5% of samples
- 35% of samples with elevated FIB did not have detectable levels of either assay suggesting additional source-types may be significant.

Keywords: Microbial source tracking, fecal contamination, fecal indicator bacteria, Mohawk River

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Introduction

Current identification methods for potential fecal contamination of a water source focus on culture-based approaches for the quantification of fecal indicator bacteria (FIB). However, FIB are not refined enough to provide information on the source type of the contamination. This is an important distinction for source identification and remediation efforts as well as risk assessments given pathogenic loads will vary depending on the contaminant source[1]. Microbial source tracking (MST) is one method which looks to use the microorganisms to identify the source of contamination. Many advances in MST have relied on gene-specific assays for qPCR to identify potential sources of fecal contamination[2]. This study focuses on the addition of MST to a concurrently funded project focused on general water quality and FIB along 10 sites in the Mohawk river watershed (Figure 1).

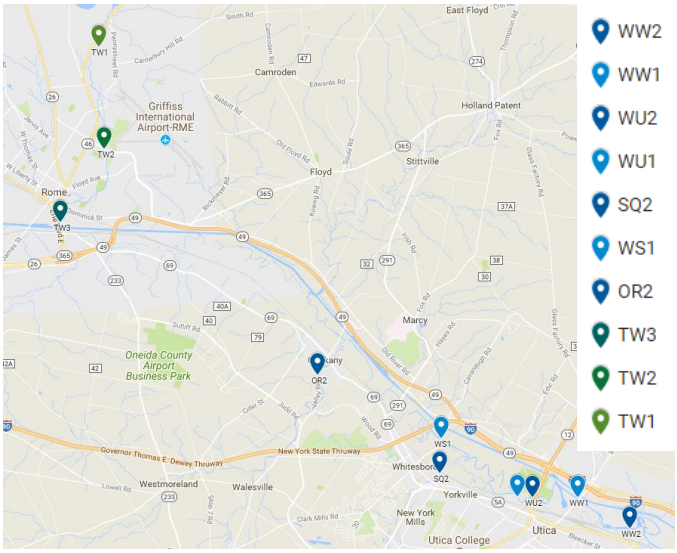

Figure 1: Location of 10 sites in the Utica-Rome region of the Mohawk river watershed.

Results & Discussion

**Raw data available by request*

<u>This section of the report represents and a preliminary analysis of the data collected. The evaluation and validation of the data is ongoing.</u>

Overall, 200 samples were collected along the western portion of the Mohawk river representing 10 sites and 20 sampling dates. As an initial step toward quality control for this report, only samples with a complete dataset and positive concentration estimates from the UV Spectrophotometer were used in the analysis. The end result was 148 samples for the analysis with between 13 and 17 at each site.

Initial preparation of the samples including the centrifugation of 300mL of initial sample and DNA extraction using the Qiagen PowerSoil kit. Table 1 shows the average, standard deviation, maximum, and minimum concentration of DNA for each site as measured using UV Spectrophotometry. Overall, centrifugation yielded usable concentrations of DNA for the analysis and represents a viable alternative to sample filtration. However, a greater starting volume is recommended due to occasional low concentrations of starting material, significant variability in amount, and the likely desire for future researchers to run multiple assays on each sample.

Table 1: Estimated DNA concentration (ng/uL)

Site	n	Ave.	Stdev	Max	Min
TW1	15	46.729	86.420	329.350	0.323
TW2	14	31.121	46.470	154.454	0.478
TW3	13	26.087	40.391	132.035	0.215
OR2	15	28.287	28.432	118.022	2.061
WS1	15	39.882	64.879	240.739	1.266
SQ2	14	37.384	51.343	200.837	1.948
WU1	17	38.580	53.831	173.893	0.583
WU2	13	18.909	34.180	129.279	0.078
WW1	16	35.820	67.531	273.171	1.469
WW2	16	27.399	57.878	233.208	0.229

Several TaqMan gene expression assays were explored at the start of the study including one that targets all bacteria (pan-bacterial) as well as more source-specific assays for humans, cows, gulls, canines, and ruminants. These assays target the ribosomal 16s gene in a region common to fecal bacteria (i.e., pan-bacterial) or a region specific to the species under investigation. Initial experiments optimized the total amount of template DNA in each qRT-PCR reaction at 2 ng for pan-bacteria, and 6 ng for all other species. All assays in these initial tests demonstrated potential for use in the region but require additional validation. Based on the starting material available and study interests, all samples were evaluated using three assays: general/all bacteria, HF183 (human), and COWM3 (cow). All 148 samples indicated the presence of bacteria based on the pan-bacterial ribosomal 16s target assay. Although this will not be discussed further in this report, the pan-bacterial signal is essential for determining

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the percent of the bacterial signal represented by species-specific assays in a future analysis.

For the initial evaluation of the data, we chose to focus on general presence / absence of the human and cow markers and the occurrence of fecal indicator bacteria E. Coli and Enterococci from the companion study. Consistent with previous studies on this portion of the Mohawk, the samples demonstrated frequent elevated FIB concentrations quantified as violations of the beach action value (BAV) set forth in the 2012 Recreational Water Quality Criteria[3]. Applied in their most conservative form here, the BAV threshold for E. coli and Enterococci is 190 CFU/100mL and 60 CFU/100mL respectively. Applying this threshold to the data, it can be seen that the BAV was violated just over 61% of the time, with slightly more frequent violations for the E. coli BAV (Table 1). This can be compared to the frequency of detection of the HF143 and CowM3 markers. The human marker was detected more frequently in the samples than the cow marker, occurring in 52% of the samples versus 5%. This result supports the general hypothesis that human sources, and in particular the CSO network in Utica discussed in the next section, is likely a source of fecal contamination in this region.

Table 2: General occurrence of BAVs and MST markers

Metric	# of samples	Percent
E. coli ≥ BAV	73	49%
Enterococci ≥ BAV	60	41%
E. coli ≥ BAV *and/or* Enterococci ≥ BAV	91	61%
HF183 present	77	52%
COWM3 present	8	5%

The data was subdivided by site to explore the occurrence of FIB and markers at the ten specific sites sampled (Table 3). The maximum in each column is highlighted in yellow. FIB violate the BAV less frequently for the three sample sites in Rome (TW1, TW2, and TW3). These three sites also demonstrated less frequent detection of the human marker HF183. The human marker was detected in 68-77% of the samples at sites along the Mohawk in the city of Utica. The three sites along tributaries and/or just prior to entering the city demonstrated slightly less frequent detection of the human marker (50%). The result is a generally increasing trend in the occurrence of the human marker following the flow of the water from the tailwaters in Rome to the eastern edge of Utica.

Unlike HF183, CowM3 was detected at low frequency along the ten sampling sites. The most frequent detection occurred at WS1 which has potential agricultural influences upstream. Given the potential for these upstream influences, it is not unreasonable to occasional detect this marker in hydrologically connected sites. Finally, both tributaries demonstrated considerably more frequent BAV violations than marker detections suggesting additional sources of fecal contamination may be present. While this cannot be fully explained at this time, it is worth noting that during the initial evaluation of all potential source markers these sites did indicate the potential presence of other sources.

Table 3: Occurrence of BAV and MST markers by site

Site	BAV E. coli	BAV Entero	BAV Any	HF183	Cow M3
TW1	13.3%	20.0%	20.0%	0.0%	0.0%
TW2	14.3%	14.3%	14.3%	42.9%	7.1%
TW3	15.4%	30.8%	30.8%	23.1%	0.0%
OR2	66.7%	40.0%	80.0%	53.3%	0.0%
WS1	53.3%	26.7%	66.7%	53.3%	13.3%
SQ2	85.7%	100%	100%	50.0%	7.1%
WU1	58.8%	52.9%	82.4%	70.6%	5.9%
WU2	53.8%	38.5%	84.6%	76.9%	7.7%
WW1	50.0%	31.3%	56.3%	75.0%	6.3%
WW2	75.0%	50.0%	75.0%	68.8%	6.3%

The general co-occurrence of FIB and marker presence / absence is shown in Table 4. Within this dataset, the most common condition for a sample was one or more of the FIB above the BAV *and* the detection of one or both of the markers. This occurred in 40% of the samples. The second most common condition occurred in 26% of the samples and represented a combination of both FIB below the BAVs and no detection of either marker. This demonstrates a general agreement between the two measures. However, approximately 22% of the samples demonstrated at least one elevated FIB above the BAV and no detection of the human or cow markers. This suggests that other sources of fecal contamination are likely present and potentially significant in the region. Finally, 12% of the samples included the detection of one of the markers but both FIB were below their respective

BAV. This is not an unexpected condition as there is no reason the markers could not occur simultaneously with lower counts of FIB.

Table 4: Co-occurrence of FIB and MST Markers

Condition	Marker	No marker	Total
Any FIB ≥ BAV	59	32	91
Both FIB < BAV	18	39	57
Total	77	71	148

Policy Implications

The analysis of this work included the comparison use of the FIB and MST. In the region of study, many of the elevated FIB samples also included the co-occurrence of the human marker. In addition, the frequency of occurrence of the markers in different geographical regions can provide guidance on what remedial actions would likely be necessary to reduce fecal contamination in the region. For example, elevated FIB in the city of Rome may be less likely to be of human origin than a similar elevated FIB in Utica. FIB or MST on their own will only provide one part of the picture but when viewed together these tools have the potential to provide a stronger picture of regional contamination issues.

Methods

The method section has been subdivided into four broad sections with brief descriptions: sample collection, bacterial concentration, DNA extraction, and source analysis via PCR.

Collection

500 mL grab samples were collected for MST at 10 locations along the western portion of the Mohawk River in Utica and Rome over 20 days in the summer of 2019 (Figure 1). Collection of samples took approximately 2 hours during which the samples were kept on ice. Samples were immediately processed upon return to the laboratory.

Bacterial Concentration

Centrifugation was chosen as a low cost, low waste method for concentration of solids within the water samples. Each sample was poured into six 50mL tubes, for a total of 300mL, and spun for initial pellet formation. After the initial round of centrifugation (3,800 x g, 15 minutes), the supernatant was removed and the formed pellets were recovered in sterile distilled water and combined in to a 1.5mL tube. This concentrated 1.5mL sample was centrifuged again at and stored at -80^{o}C after supernatant removal.

DNA Extraction and quantification

DNA was extracted from all samples using the Qiagen DNeasy PowerSoil Pro Kit according to the manufacturer's protocol (available upon request). Once the DNA was extracted, the concentration and purity of the sample was determined using the DENOIVS UV Spectrophotometer. Samples were then stored at -80^{o}C.

Source Analysis via PCR

After evaluation of DNA concentrations, samples were diluted in RNase/DNase-free sterile distilled water to a uniform concentration of 1 ng/µL, and evaluated for the presence of: general/all bacteria (2 ng total DNA), human-specific bacteria (HF183, 6 ng), and bovine-specific bacteria (COWM3, 6 ng) using commercially available TaqMan gene expression assays. PCR was conducted using the Applied Biosystems Quantstudio Real-Time PCR System. Additional validation and application of other source-specific primers is ongoing.

Outreach Comments

It is the intention of the investigators to share these findings at the Mohawk Watershed Symposium, and therefore with the community, when the opportunity returns.

Student Training

Two students were trained and participated in this grant:

- Elizabeth Haddad, Chemical Engineering student at University of Buffalo Summer 2019. Responsibilities: Collection and centrifugation of samples along with additional responsibilities for the parallel DEC funded work. Deliverable: Poster at the SUNY Poly summer undergraduate research program poster session.
- Michael Ormanoski, Civil Engineering student at SUNY Polytechnic, Fall 2020. Responsibilities: Resuspension, DNA extraction and measurement, preparation of samples for PCR. Deliverable: Final protocol summary and poster for the 2021 Spring SUNY Poly student showcase.

The project proposed was intended to be interdisciplinary. Two seniors in Biology at SUNY Poly were initially identified to complete the work during the Spring semester of 2020. Due to COVID and a campus

shut down, the work was delayed and ultimately completed in the Fall of 2020 by Michael Ormanoski a senior in the Civil Engineering program at SUNY Poly with a previous B.S in Biology from the University of Rochester.

Publications/Presentations

Elizabeth Haddad and Carolyn Rodak 2019. "An assessment of the Mohawk River Water Quality". 2019 SUNY Polytechnic Institute Summer Undergraduate Research Program (SURP) August 1st and 2nd Utica and Albany NY.

Additional presentations and publications are expected but are still in preparation.

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