**Description of Urban Stormwater Microbial Source Tracking and Antibiotic Resistance Gene Dataset**

**Overview:**

This dataset includes water quality measurements from an urban stream (Site A) and three municipal separate storm sewer system outfalls (Sites B-D) situated in a mixed-use urban catchment located in the District of Columbia, USA (**Figure 1**). The total catchment area is 61 ha with outfall drainage areas ranging from 4.69 ha (Outfall Site D) to 37.4 ha (Outfall Site C). Catchment and outfall boundaries were defined with the Spatial Analysis Hydrology tool using outfall and elevation data from the National Hydrology Dataset (<http://datagateway.nrcs.usda.gov>) combined with local stormwater transit drain and pipe data (Department of Energy and Environment, Washington, DC). Outfall drainage area residential land use ranged from 2.35 ha (Site D) to 17.1 ha (Site C). Residential metrics were defined with ArcGIS ArcMap (version 10.3; ESRI, Redlands, CA).

Map

Description automatically generated

**Figure 1:** Geographic information system (GIS) map location of urban stream, outfall sites, and residential land use assignments. The underlying street map is from OpenStreetMap (data available under the Open Database License: <https://www.openstreetmap.org/copyright>).

A total of 124 samples were collected across 31 sampling events over 12-months. Routine sampling occurred twice per month. Seven additional sampling events were conducted immediately after cumulative precipitation from a single storm exceeded 2.54 mm following a 72 h dry period based on the United States Environmental Protection Agency recommended storm event sampling guidance [1]. Urban stream and outfall samples were always collected on the same day within a short time (< 1 h). Samples were collected in sterile 1 L containers, immediately stored on ice, and transported to the laboratory (< 8 h). A field blank consisting of molecular grade water was included for each sampling day.

For each sample, 100 mL was filtered through a 0.45 µm polycarbonate filter (Fisher Scientific, Pittsburg, PA), placed in a 2 mL screw cap tube containing a silica bead mill matrix (GeneRite, North Brunswick, NJ), and stored at -80℃ (<18-months). DNA extraction was performed with the DNA-EZ RW02 kit (GeneRite) as previously described [2]. DNA was eluted with 100 µL elution buffer into low-adhesion microcentrifuge tubes and stored at 4℃ prior to qPCR amplification (< 24 h). Three method extraction blanks with molecular grade water substituted for DNA extract were performed to monitor for potential DNA contamination for each sample batch.

Genetic testing employed eleven clinically relevant ARG qPCR assays targeting *bla*CMY-2, *qnrA*, *bla*KPC, *bla*OXA-48, *bla*NDM, *bla*VIM, *mcr-1*, *mecA*, *sul1*, *tetW*, and *vanA* (**Table 1**). The HF183/BacR287 qPCR assay was used to characterize human fecal pollution levels [3-5]. The Sketa22 qPCR assay was used as a sample processing control (SPC) [6].

**Table 1:** Clinically relevant antibiotic resistance gene (ARG) qPCR assay oligonucleotide sequences.

|  |  |  |
| --- | --- | --- |
| **qPCR Target** | **Primer and Probe Sequences (5’ 🡪 3’)** | **Reference** |
| *bla*OXA-48 | F: GATTATGGTAATGAGGACATTTCGGGC  R: CATATCCATATTCATCGCAAAAAACCACAC  Probe: FAM-CCATTGGCTTCGGTCAGCATGGCTTGTTT-ZEN/IBFQ | [7] |
| *bla*KPC | F: GCAGCGGCAGCAGTTTGTTGATT  R: GTAGACGGCCAACACAATAGGTGC  Probe: FAM-CAGTCGGAGACAAAACCGGAACCTGC-ZEN/IBFQ |
| *bla*NDM | F: CCAGCAAATGGAAACTGGCGAC  R: ATCCAGTTGAGGATCTGGGCG  Probe: FAM-ACCGAATGTCTGGCAGCACACTTC-ZEN/IBFQ |
| *bla*VIM | F: TTGCTTTTGATTGATACAGCGTGGGG  R: GTACGTTGCCACCCCAGCC  Probe: FAM-TCTCGCGGAGATTGAAAAGCAAATTGGACTTCC-ZEN/IBFQ |
| *bla*CMY-2 | F: AGACGTTTAACGGCGTGTTG  R: TAAGTGCAGCAGGCGGATAC  Probe: FAM-TATCGCCCGCGGCGAAAT-ZEN/IBFQ | [8] |
| *mcr-1* | F: CATCGCGGACAATCTCGG  R: AAATCAACACAGGCTTTAGCAC  Probe: FAM-AACAGCGTGGTGATCAGTAGCAT-ZEN/IBFQ | [9] |
| *sul1* | F: CCGTTGGCCTTCCTGTAAAG  R: TTGCCGATCGCGTGAAGT  Probe: FAM-CGAGCCTTGCGGCGG-ZEN/IBFQ | [10] |
| *mecA* | F: AACCACCCAATTTGTCTGCC  R: TGATGGTATGCAACAAGTCGTAAA  Probe: FAM-CCTTGTTTCATTTTGAGTTCTGCAGTACCGG-ZEN/IBFQ | [11] |
| *tetW* | F: GCAGAGCGTGGTTCAGTCT  R: GACACCGTCTGCTTGATGATAAT  Probe: FAM-TTCGGGATAAGCTCTCCGCCGA-ZEN/IBFQ | [12] |
| *vanA* | F: CTGTGAGGTCGGTTGTGCG  R: TTTGGTCCACCTCGCCA  Probe: FAM-CAACTAACGCGGCACTGTTTCCCAAT-ZEN/IBFQ | [13] |
| *qnrA* | F: GGATGCCAGTTTCGAGGA  R: CCTGAACTCTATGCCAAAGC  Probe: FAM-CACTTCAGCTATGCCGATCTGCGCGAT-ZEN/IBFQ | [14] |

In addition to water sampling, 3,743 quality control samples, including field blanks, method blanks, and no template controls were collected and processed in an identical manner as water samples and are reported in a separate data file [Shanks\_MST ARG\_FINAL\_04152025.xlsx].

Six water quality parameters were measured directly from collected samples. Water quality parameters included turbidity (NTU), pH, conductivity (µS/cm), water temperature (℃), dissolved oxygen (mg/L), and total suspended solids (g/L). Parameters were measured with a YSI 6920 Multiparameter Water Quality Meter (Xylem Analytics, Yellow Springs, OH).

*E. coli* enumeration was performed within eight hours of sample collection with Colilert IDEXX defined substrate technologies (IDEXX Laboratories, Inc. Westbrook, ME, USA).

Precipitation was measured with a reference gauge (WH24B Wireless Weather Station, Fine Off-Set Electronics, Ltd) based on proximity to catchment (< 5 km). Cumulative precipitation of 12 h prior to a sampling event (mm) was used for all statistical analyses due to the nature and impact of precipitation events in urban catchments.

The results for antibiotic resistance gene and microbial source tracking measurements along with other water quality and precipitation datasets and a data dictionary are contained in a separate file [Shanks\_MST ARG\_FINAL\_04152025.xlsx].

**Sampling Period Included in this Data Release:** November 25, 2019 through November 30, 2020

**Point of Contact:** Orin Shanks US EPA / ORD / CEMM / WECD / BMB

**Disclaimer:** This research dataset has been reviewed in accordance with U.S. Environmental Protection Agency (U.S. EPA), Office of Research and Development, and approved for release. Mention of brand names or vendors does not constitute an endorsement of products or services by the U.S. EPA.

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