Electronic Supplementary Material (ESI) for Environmental Science: Advances. This journal is © The Royal Society of Chemistry 2022

# Supporting Information: Seasonal and spatial patterns differ between intracellular and

## extracellular antibiotic resistance genes in urban stormwater runoff

Kassidy O'Malley<sup>a</sup>, Patrick McNamara<sup>a</sup>, Walter McDonald<sup>a</sup>

<sup>a</sup>Department of Civil, Construction, and Environmental Engineering, Marquette University,

Milwaukee, WI 53233, USA

\*Corresponding author

Walter McDonald: (414) 288-2117, walter.mcdonald@marquette.edu

Department of Civil, Construction, and Environmental Engineering

Marquette University

1637 W Wisconsin Ave

Milwaukee, WI 53233 USA.

#### **Table of Content**

Stormwater Sampling	2
Streamflow Data	5
Water Quality Results	7
Correlation Analysis	11
DNA Extraction and ddPCR	16
References	17

### **Stormwater Sampling**



Fig. S1 Map of the sampling locations for this study across Milwaukee County in Wisconsin,

USA. The sites include a stormwater inlet, a stormwater outfall, the Menomonee River, and Lake

Michigan. The map was created using ArcGIS software by Ersi.



Fig. S2 Absolute gene abundance of intracellular genes by sampling location that occurred in the fall, winter, and spring under baseflow (n=9) and stormflow conditions (n=11). Error bars represent the standard deviation of the mean and statistically significant relationships between the average absolute abundance of the extracellular and intracellular genes were evaluated by Tukey's posthoc test and shown with \* (p<0.05), \*\* (p<0.01), \*\*\* (p<0.001), and \*\*\*\*(p<0.0001).

FALL WINTER

SPRING

![](_page_3_Figure_0.jpeg)

**Fig. S3** Absolute gene abundance of extracellular genes by sampling location that occurred in the fall, winter, and spring under baseflow (n=3) and stormflow conditions (n=3). Error bars represent the standard deviation of the mean and statistically significant relationships between the average absolute abundance of the extracellular and intracellular genes were evaluated by Tukey's posthoc test and shown with \* (p<0.05), \*\* (p<0.01), \*\*\* (p<0.001), and \*\*\*\*(p<0.001).

FALL WINTER SPRING

#### **Streamflow Data**

![](_page_4_Figure_1.jpeg)

![](_page_4_Figure_2.jpeg)

![](_page_5_Figure_0.jpeg)

**Fig. S4** Stream discharge recorded by USGS stream gage 04087120 during the baseflow and stormflow sampling events in the fall (A), winter (B), and spring (C).

#### Water Quality Results

![](_page_6_Figure_1.jpeg)

![](_page_7_Figure_0.jpeg)

**Figure S5.** Concentration of various metals quantified under baseline and storm conditions. Error bars represent the standard deviation of the means (n=3).

![](_page_7_Figure_2.jpeg)

Fig. S6 Air and water temperatures recorded at the time of baseline and storm sampling.

![](_page_7_Figure_4.jpeg)

Fig. S7 pH of baseline and stormflow samples. Error bars represent the standard deviation of the means (n=3).

![](_page_8_Figure_0.jpeg)

**Fig. S8** Conductivity of baseline and stormflow samples. Error bars represent the standard deviation of the means (n=3).

![](_page_8_Figure_2.jpeg)

Fig. S9 Concentration of total suspended solids (TSS) quantified in baseline and storm samples.

![](_page_9_Figure_0.jpeg)

![](_page_9_Figure_1.jpeg)

![](_page_9_Figure_2.jpeg)

**Fig. S11** Concentration of dissolved organic carbon (DOC) quantified in baseline and storm samples. Error bars represent the standard deviation of the means (n=3).

![](_page_9_Figure_4.jpeg)

**Fig. S12** Ultraviolet–visible spectrophotometry (UV-Vis) measured in baseline and storm samples.

## **Correlation Analysis**

	fall	fall	winter	winter	spring	spring
	baseflow	stormwater	baseflow	stormflow	baseflow	stormflow
i BLATEM	0.73	-0.68	-0.49	-0.26	0.62	-0.26
i ERMF	0.67	-0.46	0.67	-0.06	0.73	-0.06
i SUL1	0.83	0.98	-0.33	0.29	0.54	0.29
i TETC	-0.02	0.54	-0.59	0.34	0.49	0.34
e BLATEM	-0.09	0.85	0.45	0.32	-0.08	0.32
e ERMF	0.64	-0.46	0.23	-0.44	0.13	-0.44
e SUL1	0.56	0.04	-0.80	-0.39	0.30	-0.39
e TETC	0.50	0.70	0.31	-0.25	0.27	-0.25
e BLATEM e ERMF e SUL1 e TETC	-0.09 0.64 0.56 0.50	0.85 -0.46 0.04 0.70	0.45 0.23 -0.80 0.31	0.32 -0.44 -0.39 -0.25	-0.08 0.13 0.30 0.27	0.32 -0.44 -0.39 -0.25

**Table S1** Correlation analysis (Pearson's r) of ARGs and *intI*1 by sampling event

Bolded indicates a p-values<0.05

Table S2 Correlation analysis (Pearson's r) of ARGs and *intI*1 by sampling location

	outlet	river	lake	inlet	outlet	river	lake
	baseflow	baseflow	baseflow	storm	storm	storm	storm
i BLATEM	0.24	0.73	-0.49	0.19	0.35	-0.05	-0.56
i ERMF	0.34	0.26	0.44	0.22	0.66	0.35	0.51
i SUL1	-0.36	0.46	0.23	0.13	0.66	-0.49	0.97
i TETC	0.45	0.43	0.38	0.62	0.99	0.45	0.92
e BLATEM	-0.09	0.32	0.82	0.39	0.63	0.83	-0.35
e ERMF	0.70	0.32	0.66	0.53	0.87	0.84	-0.25
e SUL1	0.06	0.42	0.70	0.39	0.89	0.56	-0.58
e TETC	0.11	0.53	0.77	0.37	0.87	0.49	0.57

Bolded indicates a p-values<0.05

![](_page_11_Figure_0.jpeg)

Fig. S13 Pearson correlation matrix of ARGs and water quality parameters during baseflow conditions

	INTRACELLULAR					EXTRACELLULAR						
	16S						16S					
	rRNA	INTI1	BLATEM	ERMF	SUL1	TETC	rRNA	INTI1	BLATEM	ERMF	SUL1	TETC
WATER												
TEMPERATURE	-0.06	-0.34	0.39	0.12	0.23	0.46	0.05	0.39	0.33	0.63	0.08	0.42
pН	0.30	0.72	0.01	0.02	-0.18	0.06	0.03	0.19	0.02	0.14	-0.33	0.17
CONDUCTIVITY	-0.05	-0.36	-0.02	0.25	-0.08	0.16	-0.29	0.20	-0.22	-0.16	0.15	0.34
TOTAL												
SUSPENDED												
SOLIDS	0.09	0.35	0.55	0.68	0.58	0.42	-0.25	0.77	0.15	0.56	0.57	0.77
UV-VIS	0.48	0.79	0.20	0.23	-0.10	0.37	-0.20	-0.19	-0.19	-0.27	-0.64	-0.18
TOTAL												
ORGANIC												
CARBON	-0.15	0.36	-0.16	-0.42	-0.01	-0.42	0.68	0.14	0.40	0.46	0.07	0.21
TOTAL												
NITROGEN	0.13	0.38	-0.05	0.01	0.07	0.03	0.07	0.14	0.09	0.14	-0.14	0.15
TOTAL												
PHOSPHORUS	0.17	0.04	0.14	0.32	-0.01	0.30	-0.31	-0.35	-0.21	-0.34	-0.20	-0.61
CHROMIUM	-0.58	-0.71	-0.68	-0.54	-0.69	-0.44	-0.20	0.39	-0.75	0.07	-0.28	-0.56
IRON	-0.17	-0.86	-0.67	-0.60	-0.79	0.11	0.41	-0.32	-0.47	-0.17	-0.42	-0.45
NICKEL	-0.20	-0.45	-0.19	-0.65	-0.31	0.00	0.12	-0.44	0.10	-0.08	-0.36	-0.29
COPPER	-0.60	-0.92	-0.82	-0.80	-0.87	-0.14	-0.12	0.12	-0.72	-0.10	-0.21	-0.54
ZINC	-0.29	0.10	-0.28	0.34	-0.17	0.01	0.05	0.27	-0.52	-0.06	0.34	0.09
ARSENIC	-0.24	-0.72	-0.25	-0.15	-0.35	0.50	0.03	0.49	-0.38	0.31	0.21	0.20
CADMIUM	0.26	0.32	-0.28	-0.08	-0.18	-0.68	0.36	-0.71	-0.14	-0.71	-0.45	-0.62
LEAD	-0.43	-0.81	-0.90	-0.58	-0.93	-0.28	0.38	-0.22	-0.79	-0.40	-0.35	-0.69

**Table S3** Pearson's r from correlation analysis of ARGs and water quality parameters during baseflow conditions

Bolded indicates a p-values<0.05

![](_page_13_Figure_0.jpeg)

Fig. S14 Pearson correlation matrix of ARGs and water quality parameters during stormflow conditions

	INTRACELLULAR					EXTRACELLULAR						
	16S						16S					
	rRNA	INTI1	BLATEM	ERMF	SUL1	TETC	rRNA	INTI1	BLATEM	ERMF	SUL1	TETC
WATER												
TEMPERATURE	-0.27	-0.42	0.54	-0.18	-0.30	0.21	-0.39	-0.54	-0.50	0.59	-0.56	-0.42
pH	0.76	0.43	-0.36	0.20	-0.25	0.56	0.17	-0.29	-0.59	-0.59	-0.48	-0.50
CONDUCTIVITY	0.46	-0.12	-0.55	-0.11	-0.35	0.17	-0.07	-0.35	-0.30	-0.49	-0.41	-0.39
TOTAL												
SUSPENDED												
SOLIDS	-0.32	0.28	-0.46	0.10	0.20	-0.01	0.37	0.48	0.23	-0.22	-0.15	0.22
UV-VIS	-0.34	-0.05	0.08	0.19	0.11	-0.15	0.10	0.23	0.25	0.18	0.00	0.22
TOTAL												
ORGANIC												
CARBON	-0.70	-0.26	0.15	-0.13	0.32	-0.38	0.07	0.37	0.48	0.40	0.27	0.42
TOTAL												
NITROGEN	-0.49	-0.12	0.39	0.19	0.26	-0.14	0.13	0.24	0.40	0.42	0.24	0.40
TOTAL												
PHOSPHORUS	-0.54	-0.24	0.11	0.03	0.22	-0.29	0.05	0.30	0.43	0.32	0.13	0.37
CHROMIUM	-0.88	0.10	0.00	-0.14	0.10	-0.11	0.09	0.00	-0.05	0.14	-0.49	0.15
IRON	-0.15	0.31	-0.32	-0.19	0.30	0.22	0.39	0.38	0.05	0.10	-0.38	-0.03
NICKEL	-0.17	0.31	-0.36	-0.02	0.26	-0.01	0.37	0.38	0.11	-0.07	-0.25	0.09
COPPER	-0.13	-0.14	0.39	-0.29	0.01	0.34	-0.14	-0.41	-0.56	0.69	-0.44	-0.65
ZINC	-0.44	-0.35	0.49	-0.13	-0.31	-0.30	-0.40	-0.40	-0.41	0.23	-0.58	-0.14
ARSENIC	0.02	0.29	-0.51	0.10	0.22	0.10	0.42	0.45	0.31	-0.11	-0.17	0.17
CADMIUM	-0.41	0.43	-0.50	0.12	0.36	0.06	0.45	0.40	0.30	-0.14	0.08	0.19
LEAD	-0.72	0.48	-0.34	-0.15	0.44	0.01	0.46	0.39	0.16	-0.03	-0.17	0.28

**Table S4** Pearson's r from correlation analysis of ARGs and water quality parameters during stormflow conditions

Bolded indicates a p-values<0.05

#### **DNA Extraction and ddPCR**

All DNA extractions were completed within 24 hours of sampling. A one-liter water sample was vacuum filtered through a 0.22  $\mu$ m Merck Millipore Express Plus<sup>®</sup> membrane filter. iDNA was extracted from the filters via FastDNA Spin Kit manufacturer's protocol (MP Biomedicals, Santa Ana, CA). eDNA was concentrated from the filtrate by pumping the filtrate through a column containing nucleic acid adsorption particles. Following, 100 mL of an organic eluent (15 g/L NaCl, 30 g/L tryptone, 15 g/L beef extract, 3.75 g/L glycine, 0.28 g/L Na(OH), pH = 9.3 ± 0.2; autoclaved at 120°C for 20 min) was pumped through the column and collected. eDNA was extracted from the eluent via isopropanol-ethanol precipitation.

The ddPCR assays (22  $\mu$ L) consisted of a reaction mixture of QX200 ddPCR EvaGreen Supermix (Bio-Rad) (11  $\mu$ L), forward and reverse primers (250 nM each) (Table S5), diluted DNA extracts (4  $\mu$ L), and Sigma® Life Science Molecular Biology Reagent (5.9  $\mu$ L). The quantitative digital PCR experiments (dMIQE) checklist was completed for quality assurance and control <sup>1</sup>. The samples from this study averaged 14,966 positive droplets per reaction. If the number of droplets generated was <10,000 per 20  $\mu$ L PCR, the reaction was rejected <sup>2</sup>.

Gene	Annealing	Forward Primer and Reverse Primer	Reference
	Temperature		
16S rRNA	60°C	For. (5'-CCTACGGGAGGCAGCAG-3')	3
intI1	60°C	Rev. (5'-TCCACGCATCGTCAGGC-3')	4
hlatem	60°C	For. (5'-GCKGCCAACTTACTTCTGACAACG-3')	5
OTATEM	00 0	Rev. (5'- CTTTATCCGCCTCCATCCAGTCTA-3')	
ormF	60°C	For. (5'-TCGTTTTACGGGTCAGCACTT-3')	6
ermi	00 C	Rev. (5'- CAACCAAAGCTGTGTCGTTT-3')	
au11	60°C	For. (5'-CCGTTGGCCTTCCTGTAAAG -3')	7
SULI	60 C	Rev. (5'- TTGCCGATCGCGTGAAGT-3')	
4-40	60°C	For. (5'-GCGGGATATCGTCCATTCCG -3')	8
ter	00°C	Rev. (5'-GCGTAGAGGATCCACAGGACG-3')	-

Table S5 ddPCR conditions and primers

#### References

- J. F. Huggett, C. A. Foy, V. Benes, K. Emslie, J. A. Garson, R. Haynes, J. Hellemans, M. Kubista, R. D. Mueller, T. Nolan, M. W. Pfaffl, G. L. Shipley, J. Vandesompele, C. T. Wittwer and S. A. Bustin, The digital MIQE guidelines: Minimum information for publication of quantitative digital PCR experiments, *Clin. Chem.*, 2013, **59**, 892–902.
- A. B. Košir, C. Divieto, J. Pavšič, S. Pavarelli, D. Dobnik, T. Dreo, R. Bellotti, M. P. Sassi and J. Žel, Droplet volume variability as a critical factor for accuracy of absolute quantification using droplet digital PCR, *Anal. Bioanal. Chem.*, 2017, **409**, 6689–6697.
- G. Muyzer, E. C. De Waal and A. G. Uitterlinden, Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA, *Appl. Environ. Microbiol.*, 1993, **59**, 695–700.
- 4 C. Goldstein, M. D. Lee, S. Sanchez, C. Hudson, B. Phillips, B. Register, M. Grady, C. Liebert, A. O. Summers, D. G. White and J. J. Maurer, Incidence of class 1 and 2 integrases in clinical and commensal bacteria from livestock, companion animals, and exotics, *Antimicrob. Agents Chemother.*, 2001, **45**, 723–726.
- 5 E. Marti, J. Jofre and J. L. Balcazar, Prevalence of Antibiotic Resistance Genes and Bacterial Community Composition in a River Influenced by a Wastewater Treatment Plant, *PLoS One*, 2013, **8**, 1–9.
- 6 D. W. Graham, S. Olivares-Rieumont, C. W. Knapp, L. Lima, D. Werner and E. Bowen, Antibiotic resistance gene abundances associated with waste discharges to the Almendares river near Havana, Cuba, *Environ. Sci. Technol.*, 2011, **45**, 418–424.
- N. Wang, X. Yang, S. Jiao, J. Zhang, B. Ye and S. Gao, Sulfonamide-resistant bacteria and their resistance genes in soils fertilized with manures from Jiangsu province, Southeastern China, *PLoS One*, , DOI:10.1371/journal.pone.0112626.
- 8 T. B. Stanton and S. B. Humphrey, Isolation of tetracycline-resistant Megasphaera elsdenii strains with novel mosaic gene combinations of tet(O) and tet(W) from swine, *Appl. Environ. Microbiol.*, 2003, **69**, 3874–3882.