Supporting Information

A micro-geographic approach to the occurrence, fate, and risk of antibiotic residues in wastewater effluent-impacted streams across rural and sub-urban areas of Minnesota

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Text S1. Chemicals and reagents

The 25 targeted antibiotics and 10 isotopically labeled compounds used as internal standards (ISTDs) are listed in Table S1 of the Supporting Information (SI) with their respective CAS numbers, chemical structures, and supplier. Liquid chromatography−mass spectrometry grade methanol and ultrapure water (18.2 MΩ·cm at 25 °C) were used in the preparation of stock and working solutions, the solid phase extraction (SPE) protocol, and liquid chromatography with triple quadrupole tandem mass spectrometry (LC-MS/MS) analysis. For all antibiotics and ISTDs, stock solutions were prepared between 10-500 mg L^{-1} in LC-MS grade methanol and stored at −20 °C.

Text S2. Sites description and sample collection

Jewett Creek (latitude 45.141453° N, longitude 94.523591° W) and Phillips Creek (latitude 44.554018° N, longitude 93.576577° W) are low-order perennial streams (*i.e.,* second- and first-order, respectively) with catchment areas of 65 and 24 km^2, respectively, and similar land cover (~20% developed areas and ~66% agriculture).[1] The South Fork Zumbro River (latitude 44.061021° N, longitude 92.466765° W) is a fourth-order stream with a larger catchment area of 807 km^2 with developed, agriculture, and forested areas comprising 16, 72, and 9% of the watershed, respectively (see Table S2).[1]

The streams and river were selected due to the defined inputs of wastewater discharges with enough relative volume compared to the river or stream flow, which make them ideal for understanding the fate, transport, and attenuation of antibiotics. Surface water samples were collected by immersing pre-combusted 1-gallon glass bottles 10 cm below the water surface until bottles were filled. The samples were transported to the laboratory on ice and stored at 4 °C until extraction and analysis. Dissolved organic carbon (DOC) concentrations were measured as non-purgeable organic carbon by a Shimadzu TOC-L analyzer (Shimadzu; Kyoto, Japan). Anions (*e.g.,* chloride, sulfate, and nitrate) were quantified by ion exchange chromatography (Metrohm, Herisau, Switzerland), whereas total suspended solids (TSS) were analyzed according to Standard Methods.[2]

Text S3. Sample extraction, processing, and antibiotic calculations

Surface water samples were passed through pre-combusted 0.7 μm nominal pore size glass fiber filters (47 mm diameter, Millipore Sigma (Massachusetts, USA)) and acidified to pH ≤ 3.0 using sulfuric acid. Recovery corrections were applied to antibiotic concentrations to account for matrix effects and extraction performance (Text S1). Field and laboratory blanks were prepared with ultrapure water (18.2 MΩ·cm at 25 °C) and analyzed according to the same protocols, and no antibiotics were detected. The 1-gallon water samples were split into triplicate 1-L subsamples. All subsamples were spiked with 40 μL of a 5 mg L^{-1} surrogate stock solution. One of the sub-samples was also spiked with 20 μL of 5 mg L^{-1} of antibiotic solution of all target compounds as a standard addition to estimate the absolute recovery. All subsamples were stored in a cold room (4 °C) overnight for equilibration. Field and lab blank were also treated as field samples in the processing step. Solid-phase extraction (SPE) was carried out using a HLB cartridge (200 mg, 6 cm^3, 30 μm). Conditioning steps included adding 5 mL of methanol, 5 mL of ultrapure water (18.2 MΩ·cm at 25 °C), and 5 mL of ultrapure water acidified to pH ≤ 3.0 before extracting the 1-L samples. Each 1-L sample was loaded onto the cartridges using a SPE manifold at a rate not exceeding 15 mL min^{-1}. After sample loading, the cartridge was rinsed with 6 mL of ultrapure water and then dry vacuum for about 1 minute. Each SPE cartridge was then eluted by gravity with 13 mL of methanol. The extract was reduced to dryness with pure nitrogen gas within a 40 °C water bath. The residues were reconstituted in 490 μL of a 10% methanol in ultrapure water solution and 10 μL of internal standard (ISTD) solution mixture with each compound at 2 mg L^{-1} to give a total of 500 μL of solution and ISTD final concentration of 40 μg L^{-1}. The solution was vortexed for 15 seconds and sonicated for 10 min, with the process being repeated three times. The extracts were then filtered through a 0.4 um GHP syringe filter (Whatman, 13 mm) into an HPLC vial.

Analytes were measured with a LC-MS/MS (1260 Infinity II LC with 6470 MS/MS; Agilent Technologies, Santa Clara, CA, USA) equipped with an Agilent Jet Stream Technology electrospray

ionization source, operating in positive ion mode for analysis of antibiotics. A Waters Xselect CSH C18 (3.5 µm, 130 Å, 50 × 2.1 mm) column was used to separate the antibiotics. A gradient method was used with (A) 0.1% formic acid in Milli-Q ultrapure water as mobile phase and (B) methanol. The initial conditions were set at 2% B with 98% A. A linear gradient was then applied to reach 100% B over 11.5 minutes. The composition was held at 100% B until 18 minutes, after which it was returned to the starting condition of 2% B by 18.5 minutes. A re-equilibration period of 4.5 minutes was incorporated following each sample. The gas temperature was 300 °C, gas flow was 5 L min^{-1}, nebulizer was 45 psi, sheath gas temperature was 250 °C, sheath gas flow 11 L min^{-1}, capillary was 3500 V, and the nozzle voltage was 500 V. For the MS/MS detection, the instrument was operated in dynamic multiple reaction monitoring (DMRM) mode, selecting two MS/MS fragments that yielded the highest instrumental responses for compound confirmation. The precursor ions, product ions, fragmentor voltage, retention time, and collision energies of all the antibiotics, surrogates, and internal standards are listed in Table S4.

A calibration curve of eight standards comprised of all target antibiotics and surrogates ranging from 0.5 to 500 µg L^{-1} and 40 µg L^{-1} of ISTDs was prepared for each batch. Calculation of antibiotic concentrations in water samples accounted for the absolute recovery determined from the standard addition's strategy. The method detection limits (MDLs) for antibiotics ranged from 1 to 3 ng L^{-1} (bottle concentration); additionally, method quantitation limits (MQLs) were calculated as 3.3× the corresponding MDL value. For each sub-sample processed in triplicate, the absolute recovery was calculated by deducting the mean concentration found in the two replicates, which were not spiked with the target antibiotics, from the concentration in the spiked third replicate. This resultant figure was then normalized to the amount of target antibiotics initially added to the spiked replicate. Each step to calculate the final concentration of antibiotics were determined with Equations S1-S5.

$$A_{R,i} = \frac{P_i}{P_{ISTD,i}} \qquad \text{Eq. S1}$$

Where $A_{R,i}$ is the absolute response of each target antibiotic, P_i is the peak response of the target analyte, and P_{ISTDi} is the peak response of the ISTD used in each target analyte.

The concentration of a target antibiotic in the HPLC vial $C_{vial,i}$ in μg L^{-1} was calculated as follows:

$$C_{vial,i} = \frac{A_{R,i} - I_i}{S_i} \quad \text{Eq. S2}$$

Where I_i and S_i are the intercept and slope, respectively, of the linear regression obtained from the calibration curve of each target analyte.

The concentration of a target antibiotic in the sample ($C_{W,i}$) in ng L^{-1} was then calculated using the concentration factor of the SPE protocol.

$$C_{W,i} = \frac{C_{vial,i}}{V_{sample}/V_{vial}} \times 1000 \quad \text{Eq. S3}$$

In Eq. S3, V_{vial} is the final volume of the extract after SPE in mL (usually 0.5 mL), and $V_{sample}$is the volume of the sub-sample that went through SPE in mL, which was always 1000 mL.

Then, the final corrected concentration in the sample ($C_{W,corrected,i}$) in ng L^{-1} (Eq. S4) was calculated by dividing $C_{W,i}$ by the absolute recovery $R_{A,i}$ (Eq. S5). $R_{A,i}$ was calculated by subtracting the concentration of the target analyte in two replicated samples that were not spiked ($C_{vial,1}$: $C_{vial,2}$) from the concentration of the target analyte in the sample that was spiked ($C_{vial,3}$) and divided by the concentration of the actual target antibiotic in the spiked sample (C_{Spiked}) which was always 200 μg L^{-1}in the vial.

$$C_{W,corrected,i} = \frac{C_{W,i}}{R_{A,i}} \quad \text{Eq. S4}$$

$$R_{A,i} = \frac{C_{vial,3} - average(C_{vial,1}: C_{vial,2})}{C_{Spiked}} \quad \text{Eq. S5}$$

Text S4. Surface float method to estimate stream flow

Briefly, at each sampling site, a 10 – 50-m long section of the stream with uniform width was selected. Then, the cross-section, depth, and width of the stream were measured using a measuring tape. The depth of the water was measured at 1-foot intervals across the stream. The cross-sectional area was calculated for each interval by multiplying the measured depth by the interval, and then the sum of that calculation was used as the total cross-sectional area for that section of the stream. All measurements were done three times per sampling event in each site. For the stream flow velocity, a plastic ping pong ball was dropped at the beginning of the long section of the stream, and the time to travel a distance was measured. Then, the average surface velocity was calculated by averaging the measured times and dividing that by distance. Finally, the stream flow was calculated using Equation S6.

$$Q = A \times v \times a \qquad \text{Eq. S6}$$

Where Q is the stream flow in $m^3\ s^{-1}$, A is the cross-sectional area (m^2), v is the mean stream flow velocity of the surface water ($m\ s^{-1}$), and a is the velocity correction factor of 0.80 following standard protocols.[3]

Text S5. Normalized-population mass loading emission and resistance and ecological risk assessment

The normalized-population mass loading emission (NPMLE) was calculated by multiplying the antibiotic concentration in the DWW site for Jewett Creek and South Fork Zumbro River by the stream flow and dividing by the population of the city where samples were collected (Eq. S7). For Phillips Creek, NPMLE was calculated similarly to the two other sites, however, we used the concentration in DWS1 instead of DWW; this distinction is made because we wanted to understand the mass loading that is already in the stream in addition to that contributed by the wastewater effluent. This was then multiplied by the estimated stream flow:

$$\text{NPMLE} = \frac{C_i \times F \times 86{,}400 \times 10^{-3}}{P/1{,}000} \qquad \text{Eq. S7}$$

where NPMLE is the mass loading emission of each antibiotic in mg of antibiotic emitted per day per 1,000 inhabitants; C_i is the antibiotic concentration in DWW or DWS1 in ng L^{-1}; F is the streamflow in $m^3\ s^{-1}$; and P is the population of each city, which was retrieved from the United States Census Bureau.[4] Data on streamflow at DWW and DWS1 for all sites is presented in Table S5.

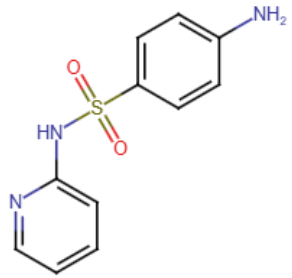
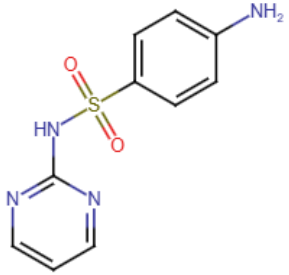
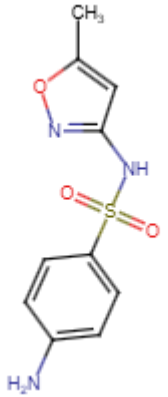
The resistance and ecological risk assessment was calculated using the risk quotient (RQ) to estimate the potential risk of antibiotics along the receiving waters. The RQ was calculated using Equation S8.

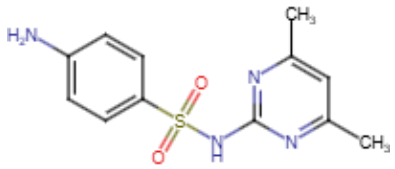
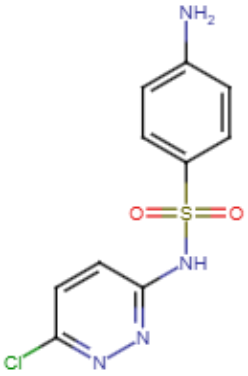
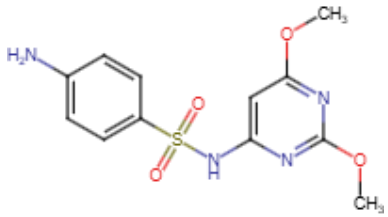
$$RQ = \frac{MEC_i}{PNEC_i} \qquad \text{Eq. S8}$$

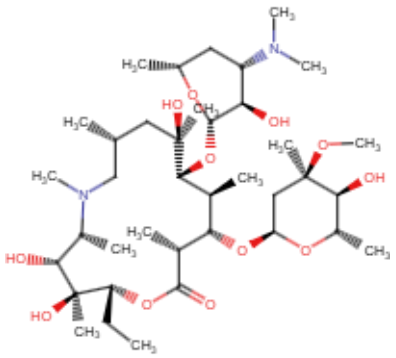
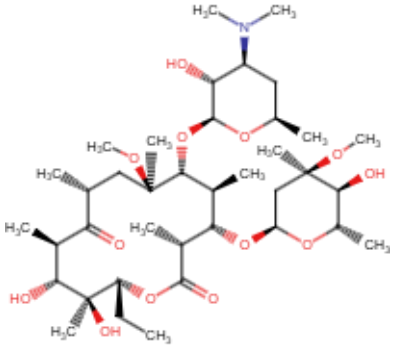
Where MEC_i is the measured environmental concentration of the targeted antibiotic in DWW, DWS1, DWS2, and DWS3, and $PNEC_i$ is the predicted no-effect concentration of the targeted antibiotic. Two RQs were estimated. The first one is the RQ for the promotion of AMR (RQ_{AMR}) which uses PNECs calculated using the minimum inhibitory concentration expected to be protective of antimicrobial resistance promotion reported by Bengtsson-Palme and Larsson [5]. The second is the RQ based on eco-toxicology for species (RQ_{ENV}) which uses PNEC data gathered by the AMR Industry Alliance[6, 7] and

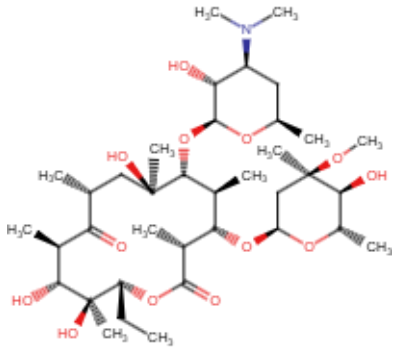
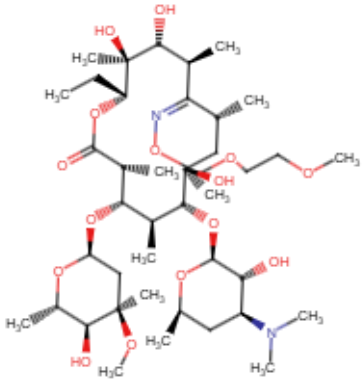
reviewed literature; [8, 9] these values are intended to be protective of ecological species. PNEC values are presented in Table S8 for the most detected antibiotics in this study. The RQ calculated values were categorized into four levels: no risk (RQ<0.01), low risk (0.01<RQ<0.1), moderate risk (0.1<RQ<1), and high risk (RQ>1).

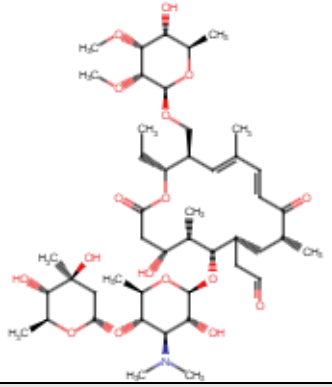
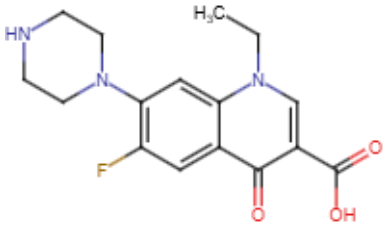
Table S1. Information about the targeted antibiotics analyzed in this study.

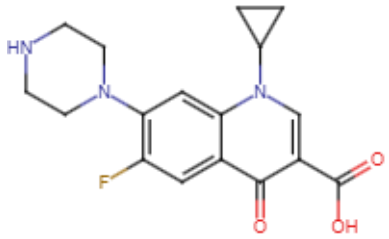
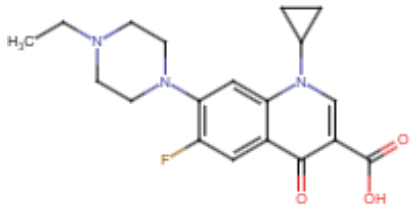
List of antibiotics	Acronymn	CAS number	Chemical Structure [a]	Supplier
Sulfonamides				
Sulfapyridine	SPD	144-83-2		Sigma-Aldrich (St. Louis, MO, USA)
Sulfadiazine	SDZ	68-35-9		Tokyo Chemical Industry (Portland, OR, USA)
Sulfamethoxazole	SMX	723-46-6		Chem-Impex Int'l. Inc. (Wood Dale, IL, USA)

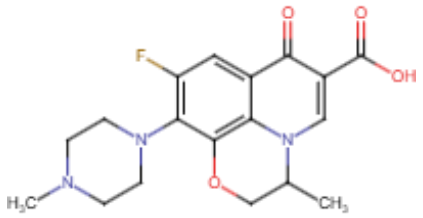
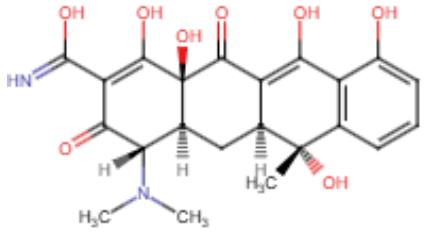
List of antibiotics	Acronymn	CAS number	Chemical Structure [a]	Supplier
Sulfamethazine	SMZ	57-68-1		Thermo Fisher Scientific (Waltham, MA, USA)
Sulfachlorpyridazine	SCP	80-32-0		Sigma-Aldrich (St. Louis, MO, USA)
Sulfadimethoxine	SDMX	122-11-2		Sigma-Aldrich (St. Louis, MO, USA)

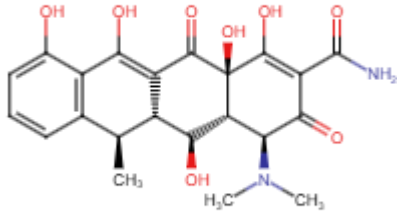
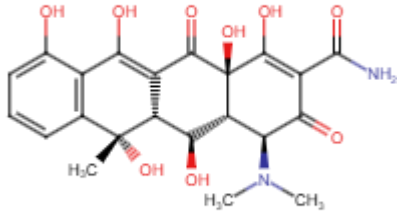
List of antibiotics	Acronymn	CAS number	Chemical Structure [a]	Supplier
Macrolides				
Azithromycin	AZMC	117772-70-0		Tokyo Chemical Industry (Portland, OR, USA)
Clarithromycin	CRMC	81103-11		Tokyo Chemical Industry (Portland, OR, USA)

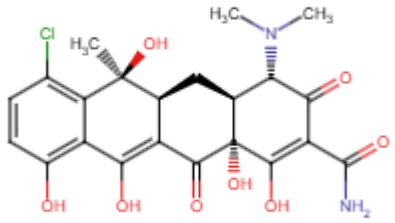
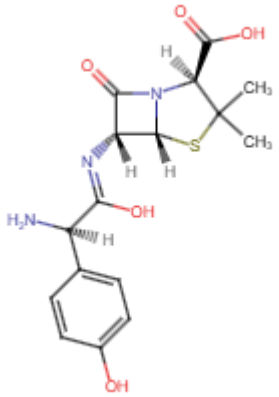
List of antibiotics	Acronymn	CAS number	Chemical Structure [a]	Supplier
Erythromycin	ERMC	114-07-8		Santa Cruz Biotechnology, Inc (Dallas, TX, USA)
Roxithromycin	RXMC	80214-83-1		Sigma-Aldrich (St. Louis, MO, USA)

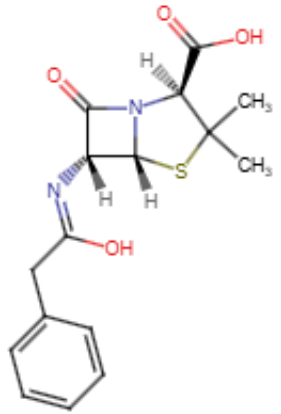
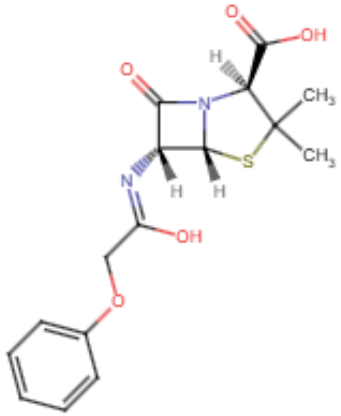
List of antibiotics	Acronymn	CAS number	Chemical Structure [a]	Supplier
Tylosin	TYL	1401-69-0		Fluka
Fluoroquinolones				
Norfloxacin	NFC	70458-96-7		Sigma-Aldrich (St. Louis, MO, USA)

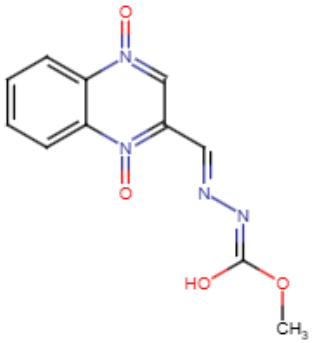
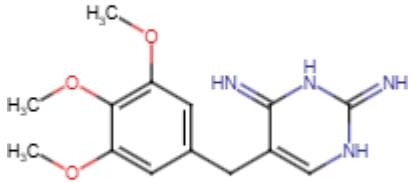
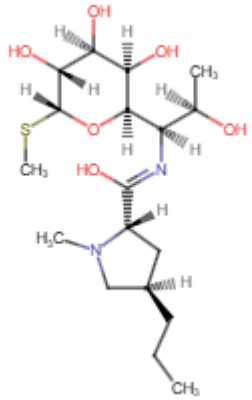
List of antibiotics	Acronymn	CAS number	Chemical Structure [a]	Supplier
Ciprofloxacin	CFC	85721-33-1		Sigma-Aldrich (St. Louis, MO, USA)
Enrofloxacin	EFC	93106-60-6		Tokyo Chemical Industry (Portland, OR, USA)

List of antibiotics	Acronymn	CAS number	Chemical Structure [a]	Supplier
Ofloxacin	OFC	82419-36-1		Acros Organic (NJ, USA)
Tetracyclines				
Tetracycline	TCC	64-75-5		Research Product International (Mt Prospect, IL, USA)

List of antibiotics	Acronymn	CAS number	Chemical Structure [a]	Supplier
Doxycycline	DXC	24390-14-5		Sigma-Aldrich (St. Louis, MO, USA)
Oxytetracycline	OTC	2058-46-0		Research Product International (Mt Prospect, IL, USA)

List of antibiotics	Acronymn	CAS number	Chemical Structure [a]	Supplier
Chlortetracycline	CTC	64-72-2		Sigma-Aldrich (St. Louis, MO, USA)
β-Lactams				
Amoxicillin	AMX	26787-78-0		Acros Organic (NJ, USA)

List of antibiotics	Acronymn	CAS number	Chemical Structure [a]	Supplier
Penicillin G	PEN-G	69-57-8		Acros Organic (NJ, USA)
Penicillin V	PEN-V	132-98-9		Acros Organic (NJ, USA)
Others				

List of antibiotics	Acronymn	CAS number	Chemical Structure [a]	Supplier
Carbadox	CBX	6804-07-5		Sigma-Aldrich (St. Louis, MO, USA)
Trimethoprim	TMP	738-70-5		Acros Organic (NJ, USA)
Lincomycin	LCM	859-18-7		Sigma-Aldrich (St. Louis, MO, USA)

List of antibiotics	Acronymn	CAS number	Chemical Structure [a]	Supplier
Internal Standards				
Sulfamethoxazole-$^{13}C_6$	SMX-$^{13}C_6$	1196157-90-0	-----[b]	MedChem Express (Monmouth Junction, NJ, USA)
Azithromycin-^{13}C-d_3	AZMC-^{13}C-d_3	2750534-82-6	-----[b]	Cayman Chemical Company (Ann Harbor, MI, USA)
Ciprofloxacin-d_8	CFC-d_8	1216659-54-9	-----[b]	Cayman Chemical Company (Ann Harbor, MI, USA)
Norfloxacin-d_5	NFC-d_5	1015856-57-1	-----[b]	Sigma-Aldrich (St. Louis, MO, USA)
Ofloxacin-d_3	OFC-d_3	1173147-91-5	-----[b]	MedChem Express (Monmouth Junction, NJ, USA)
4-epi-tetracycline-d_6	4-epi-TCC-d_6	79-85-6	-----[b]	Santa Cruz Biotechnology, Inc (Dallas, TX, USA)
Amoxicillin-d_4	AMX-d_4	26787-78-0	-----[b]	Cayman Chemical Company (Ann Harbor, MI, USA)
Penicillin V-d_5	PEN V-d_5	1356837-87-0	-----[b]	Cayman Chemical Company (Ann Harbor, MI, USA)
Trimethoprim-d_9	TMP-d_9	1189460-62-5	-----[b]	MedChem Express (Monmouth Junction, NJ, USA)
Simeton	SIM	673-04-1	-----[b]	Sigma-Aldrich (St. Louis, MO, USA)
Surrogates				
Nalidixic acid	NDA	389-08-2	-----[b]	Sigma-Aldrich (St. Louis, MO, USA)
Demeclocycline	DMC	127-33-3	-----[b]	Santa Cruz Biotechnology, Inc (Dallas, TX, USA)
Clinafloxacin	CFXC	105956-97-6	-----[b]	Santa Cruz Biotechnology, Inc (Dallas, TX, USA)

[a] Chemical structures of antibiotics were drawn in a Chemical Sketch Tool by Chemaxon at the Protein Data Bank website: https://www.rcsb.org/chemical-sketch
[b] Chemical structures were only presented for targeted antibiotic compounds.

Table S2. Name, location, stream order, sub-catchment area, distance from upstream, and land cover percentage of each stream.

Stream	Location	Stream order (Strahler method [10])	Sub-catchment area (km^2)	Distance from upstream (UPS) to downstream sites	Developed areas (%)[1]	Agriculture (%)[1]	Forested areas (%)[1]
Jewett Creek	Litchfield, MN	2nd	65	DWW: 300 m DWS1: 975 m DWS2: 1008 m DWS3: 4103 m	19.8	66.8	2.7
Philipps Creek	New Prague, MN	1st	24	DWS1: 906 m DWS2: 945 m DWS3: 1311 m	24.6	66.2	6.4
South Fork Zumbro River	Rochester, MN	4th	807	DWW: 500 m DWS1: 849 m DWS2: 1110 m DWS3: 2765 m	15.8	71.9	9.1

Table S3. Sampling dates and sample identification for Jewett Creek (JC), Philipps Creek (PC), and South Fork Zumbro River (ZR) in MN during the spring and summer of 2023 and 2024.

Year	Site	Sample Identification	Sampling date
2023	Jewett Creek	JC-2023-A	08/07/2023
		JC-2023-B	09/06/2023
		JC-2023-C	09/22/2023
		JC-2023-D	10/11/2023
	Philipps Creek	PC-2023-A	08/10/2023
		PC-2023-B	09/12/2023
		PC-2023-C	09/28/2023
		PC-2023-D	10/18/2023
	South Fork Zumbro River	ZR-2023-A	06/29/2023
		ZR-2023-B	07/25/2023
		ZR-2023-C	08/23/2023
2024	Jewett Creek	JC-2024-A	05/14/2024
		JC-2024-B	06/05/2024
		JC-2024-C	07/02/2024
	Philipps Creek	PC-2024-A	05/29/2024
		PC-2024-B	06/11/2024
		PC-2024-C	07/09/2024
	South Fork Zumbro River	ZR-2024-A	07/18/2024
		ZR-2024-B	07/29/2024
		ZR-2024-C	08/05/2024

Table S4. LC-MS/MS parameters for the targeted antibiotics, internal standards, and surrogates.

Antibiotic	Acronym	MW [a]	Mode	Frag [b]	Precursor	MS-1 [c]	CE-1 [d]	MS-2 [c]	CE-2 [d]	RT [e]	ISTD [f]
Sulfonamides											
Sulfapyridine	SPD	249.3	+	91	250.1	156.0	17	108.1	28	3.14	SMX-$^{13}C_6$
Sulfadiazine	SDZ	250.3	+	96	251.1	156.0	16	108.1	28	2.58	SMX-$^{13}C_6$
Sulfamethoxazole	SMX	253.3	+	101	254.1	92.1	32	156.0	28	5.17	SMX-$^{13}C_6$
Sulfamethazine [f]	SMZ	278.3	+	127	279.1	186.1	16	156.0	20	4.34	SMZ-$^{13}C_6$
Sulfachlorpyridazine	SCP	284.0	+	111	285.0	156.1	16	92.1	36	4.99	SMX-$^{13}C_6$
Sulfadimethoxine [f]	SDMX	310.3	+	97	311.1	156.1	24	92.1	36	7.39	SMX-$^{13}C_6$
Sulfamethoxazole-$^{13}C_6$ [f]	SMX-$^{13}C_6$	259.2	+	102	260.2	98.0	30	161.9	16	5.17	-
Macrolides											
Azithromycin	AZMC	749.5	+	175	749.5	158.0	30	591.4	30	10.57	AZMC-^{13}C-d_3
Clarithromycin	CRMC	748.0	+	123	748.5	590.3	20	158.1	28	10.58	AZMC-^{13}C-d_3
Erythromycin	ERMC	733.9	+	163	734.4	158.2	30	576.4	20	9.61	AZMC-^{13}C-d_3
Roxithromycin	RXMC	836.5	+	169	837.5	158.1	30	679.5	20	10.70	AZMC-^{13}C-d_3
Tylosin [g]	TYL	916.1	+	220	916.5	174.1	30	772.5	30	9.96	AZMC-^{13}C-d_3
Azithromycin-^{13}C-d_3 [f]	AZMC-^{13}C-d_3	752.5	+	170	753.5	595.4	30	162.1	30	6.80	-
Fluoroquinolones											
Norfloxacin [g]	NFC	319.3	+	117	320.1	302.1	24	276.1	16	3.98	NFC-d_5
Ofloxacin	OFC	361.4	+	184	362.1	318.1	20	261.1	30	3.79	OFC-d_3
Enrofloxacin [g]	EFC	359.4	+	174	360.1	342.1	24	316.2	20	4.51	CFC-d_8
Ciprofloxacin	CFC	331.4	+	117	332.1	314.1	24	231.1	30	4.23	CFC-d_8
Ciprofloxacin-d_8 [f]	CFC-d_8	339.0	+	128	340.4	322.1	20	235.0	30	4.00	-
Norfloxacin-d_5 [f]	NFC-d_5	324.0	+	135	325.1	307.1	24	281.1	16	3.96	-
Ofloxacin-d_3 [f]	OFC-d_3	364.4	+	128	365.4	321.1	20	261.0	30	3.60	-
Tetracyclines											
Tetracycline [g]	TCC	444.4	+	111	445.1	410.1	20	427.1	12	3.94	4-epi-TCC-d_6
Doxycycline [g]	DXC	444.4	+	122	445.1	428.2	20	154.0	32	6.89	4-epi-TCC-d_6
Oxytetracycline [g]	OTC	460.4	+	116	461.1	426.1	20	443.1	12	4.21	4-epi-TCC-d_6
Chlortetracycline [g]	CTC	478.0	+	112	479.1	444.1	24	462.1	16	5.60	4-epi-TCC-d_6

Antibiotic	Acronym	MW [a]	Mode	Frag [b]	Precursor	MS-1 [c]	CE-1 [d]	MS-2 [c]	CE-2 [d]	RT [e]	ISTD [f]
4-epi-tetracycline-d_6 [f]	4-epi-TCC-d_6	339.0	+	132	340.4	322.1	20	235.0	30	2.65	-
β-lactams											
Amoxicillin	AMX	365.4	+	96	366.2	349.2	4	114.0	20	1.81	AMX-d_4
Penicillin G	PEN G	334.4	+	106	335.1	160.1	8	176.1	12	10.18	PEN V-d_5
Penicillin V	PEN V	350.4	+	184	351.1	229.1	16	257.1	12	10.41	PEN V-d_5
Amoxicillin-d_4 [f]	AMX-d_4	369.4	+	102	371.0	354.2	4	115.0	20	1.68	-
Penicillin V-d_5 [f]	PEN V-d_5	355.0	+	118	356.0	160.0	8	113.9	30	10.64	-
Others											
Carbadox [g]	CBX	262.2	+	122	263.1	231.1	12	130.1	24	5.81	SIM
Trimethoprim	TMP	290.3	+	154	291.1	230.1	28	261.1	28	2.97	TMP-d_9
Lincomycin	LCM	406.5	+	143	407.3	126.2	28	359.2	20	2.40	AMX-d_4
Trimethoprim-d_9 [f]	TMP-d_9	299.4	+	164	300.4	234.0	28	264.1	28	2.72	-
Simeton [f]	SIM										
Surrogates											
Sulfamethazine-$^{13}C_6$	SMZ-$^{13}C_6$	284.2	+	117	285.1	186.1	16	124.1	28	4.35	SMX-$^{13}C_6$
Nalidixic acid	NDA	232.2	+	81	233.2	187.0	21	159.0	23	8.99	PEN V-d_5
Demeclocycline	DMC	464.8	+	122	465.1	448.1	16	430.1	24	4.65	4-epi-TCC-d_6

a: MW, molecular weight (g/mol)
b: Fragmentor voltage (V)
c: MS-1 was used for quantitation, and MS-2 was used for confirmation
d: CE, collision energy (V)
e: RT, retention time (min)
f: ISTD, internal standard used for quantitation
g: antibiotics approved for animal use
h: only one product ion

Table S5. Stream velocity, mean flow, and standard deviation (STD) for Jewett Creek and Philipps Creek estimated by the surface float method.

Site	Sample Identification	Sampling date	Flow velocity (m s^{-1})	Flow rate ± STD (m^3 s^{-1})
Jewett Creek	JC-2023-A	08/07/2023	----- [a]	----- [b]
	JC-2023-B	09/06/2023	----- [a]	----- [b]
	JC-2023-C	09/22/2023	0.4094	2.130 ± 1.132
	JC-2023-D	10/11/2023	----- [a]	----- [b]
	JC-2024-A	05/14/2024	0.3406	15.331 ± 5.516
	JC-2024-B	06/05/2024	----- [a]	----- [b]
	JC-2024-C	07/02/2024	0.3402	18.937 ± 11.284
Philipps Creek	PC-2023-A	08/10/2023		----- [b]
	PC-2023-B	09/12/2023	0.0992	0.116 ± 0.038
	PC-2023-C	09/28/2023	0.1547	0.192 ± 0.075
	PC-2023-D	10/18/2023	----- [a]	----- [b]
	PC-2024-A	05/29/2024	0.3129	5.793 ± 1.339
	PC-2024-B	06/11/2024	0.4311	4.183 ± 1.109
	PC-2024-C	07/09/2024	0.3131	5.307 ± 1.647
South Fork Zumbro River [c]	ZR-2023-A	06/29/2023	----- [c]	2.945
	ZR-2023-B	07/25/2023	----- [c]	1.894
	ZR-2023-C	08/23/2023	----- [c]	1.320
	ZR-2024-A	07/18/2024	----- [c]	12.289
	ZR-2024-B	07/29/2024	----- [c]	4.616
	ZR-2024-C	08/05/2024	----- [c]	6.994

[a] Flow velocity was not calculated due to weather conditions. However, for the in-stream attenuation constants rate calculations in those dates, an estimated of 0.40 and 0.34 m s^{-1} was used for Jewett Creek in 2023 and 2024, respectively, and 0.10 m s^{-1} for Philipps Creek in 2023.

[b] Stream flow and velocity were not calculated due to weather conditions. However, for the normalized-population mass loading emission in those dates, an estimated of 2.130 and 15. 331 m^3 s^{-1} was used for Jewett Creek in 2023 and 2024, respectively, and 0.100 m^3 s^{-1} for Philipps Creek in 2023.

[b] For the South Fork Zumbro River, stream flow was obtained from the USGS gage station 05372995.[11]; therefore, no flow velocity were registered in that site.

Table S6. Daily flow rate of the WWTP effluent at the dates the samples were collected.

Site	Sampling date	Flow rate (m^3 s^{-1})
Jewett Creek	08/07/2023	0.0736[a]
	09/06/2023	0.0745[a]
	09/22/2023	0.0760[a]
	10/11/2023	0.0755[a]
	05/14/2024	0.0826[a]
	06/05/2024	0.0949[a]
	07/02/2024	0.0907[a]
Philipps Creek	08/10/2023	0.037[b]
	09/12/2023	0.028[b]
	09/28/2023	0.027[b]
	10/18/2023	0.025[b]
	05/29/2024	0.048[b]
	06/11/2024	0.053[b]
	07/09/2024	0.070[b]

[a] Daily flow rate obtained from WWTP facility in Litchfield, MN
[b] Daily flow rate obtained from WWTP facility in New Prague, MN.

Table S7. Water quality parameters measured in stream water samples collected in 2024. Sampling dates and times are presented, as well as the average depth of sites stretches for Jewett Creek and Philipps Creek. Dissolved organic carbon (DOC), total suspended solids (TSS), and anions are presented in units of mg L^{-1}. STD is the standard deviation of the DOC concentration.

Sample ID	Site ID	Sampling date	Sampling time (US central time)	DOC (mg/L)	DOC STD (mg/L)	TSS (mg/L)	Nitrate (mg/L)	Chloride (mg/L)	Sulfate (mg/L)	Mean stream depth (m)
JC-2024-A	UPS	5/14/2024	10:11 AM	68.5	0.3	3.2	<5	35	23	0.70
	DWW		11:21 AM	8.3	0.1	7.6	63.	1134	25	0.39
	DWS1		11:38 AM	15.5	0.2	5.3	28	75	32	0.51
	DWS2		12:00 PM	48.1	0.4	7.1	35	85	25	0.51
	DWS3		12:45 PM	13.4	0.1	4.4	37	85	24	0.34
JC-2024-B	UPS	6/5/2024	10:29 AM	89.8	0.3	3.9	<5	27	13	---[a]
	DWW		10:47 AM	134.0	1.5	3.3	116	281	17	---[a]
	DWS1		11:35 AM	179.7	0.4	9.2	<5	36	19	---[a]
	DWS2		12:00 PM	163.7	0.2	6.0	<5	60	13	---[a]
	DWS3		12:16 PM	79.5	0.2	7.5	<5	58	13	---[a]
JC-2024-C	UPS	7/2/2024	10:17 AM	55.1	0.5	2.0	<5	31	12	0.74
	DWW		11:30 AM	51.9	0.4	15.2	<5	43	13	0.43
	DWS1		12:00 PM	84.6	0.8	4.8	25	70	21	---[a]
	DWS2		12:15 PM	96.7	0.9	11.6	9	68	17	---[a]
	DWS3		12:55 PM	9.2	0.1	26.3	11	66	15	---[a]
PC-2024-A	UPS	5/29/2024	9:53 AM	74.9	0.9	6.9	56	63	38	0.22
	EFF		9:30 AM	117.3	0.3	0.4	100	518	N/A	---[a]
	DWS1		10:25 AM	60.1	0.4	0.8	49	131	46	0.50
	DWS2		10:40 AM	83.6	0.2	4.1	46	131	46	0.44
	DWS3		11:15 AM	61.2	0.7	3.2	56	130	48	0.38
PC-2024-B	UPS	6/11/2024	10:15 AM	11.2	0.1	10.1	57	53	N/A	0.21
	EFF		9:48 AM	56.2	0.6	2.2	106	628	121	---[a]
	DWS1		10:34 AM	12.5	0.2	13.6	57	133	42	0.59
	DWS2		10:51 AM	15.9	0.1	13.9	45	136	44	0.59
	DWS3		11:23 AM	86.4	0.5	12.9	61	136	43	0.31
PC-2024-C	UPS	7/9/2024	10:00 AM	9.9	0.2	8.0	46	53	30	0.23
	EFF		9:25 AM	11.0	0.1	2.0	104	481	103	---[a]
	DWS1		10:35 AM	15.2	0.2	20.0	51	137	37	0.55
	DWS2		10:45 AM	10.1	0.3	27.0	52	147	38	0.55
	DWS3		11:00 AM	10.7	0.2	18.0	50	137	39	0.35
ZR-2024-A	UPS	7/18/2024	12:45 PM	24.8	0.1	18.7	45	45	28	---[b]
	DWW		12:57 PM	11.2	0.2	10.1	119	439	77	---[b]
	DWS1		1:10 PM	7.9	0.1	21.9	54	79	31	---[b]

	DWS2		1:15 PM	10.4	0.2	21.3	55	71	30	---[b]
	DWS3		1:21 PM	33.7	0.4	23.0	53	61	29	---[b]
ZR-2024-B	UPS	7/29/2024	12:50 PM	21.6	0.3	17.4	28	60	32	---[b]
	DWW		1:10 PM	14.5	0.1	4.7	169	387	98	---[b]
	DWS1		1:33 PM	18.4	0.1	7.2	40	90	37	---[b]
	DWS2		1:41 PM	5.0	0.1	9.0	38	79	35	---[b]
	DWS3		1:55 PM	17.6	0.2	8.4	48	110	41	---[b]
ZR-2024-C	UPS	8/5/2024	12:59 PM	4.8	0.1	4.1	24	62	31	---[b]
	DWW		1:17 PM	30.3	0.1	8.4	171	373	100	---[b]
	DWS1		1:26 AM	34.1	0.3	10.0	37	96	39	---[b]
	DWS2		1:35 PM	32.7	0.3	487.3	31	80	36	---[b]
	DWS3		1:47 PM	14.5	0.1	333.5	57	149	51	---[b]

[a] Depth was not measured due to weather conditions.
[a] Depth was not measured at the South Fork Zumbro River because stream water samples were collected from a canoe.

Table S8. Predicted no effect concentration (PNEC) values of ten targeted antibiotic based on eco-toxicology for species ($PNEC_{ENV}$) and calculated using the minimum inhibitory concentration expected to be protective of antimicrobial resistance promotion ($PNEC_{AMR}$).

Antibiotic	Acronym	$PNEC_{ENV}$ (µg/L)	$PNEC_{AMR}$ (µg/L)
Amoxicillin	AMX	0.57[a]	0.25 [c]
Azithromycin	AZMC	0.03 [a]	0.25 [c]
Ciprofloxacin	CFC	0.45 [a]	0.064 [c]
Clarithromycin	CRMC	0.25 [a]	0.25 [c]
Ofloxacin	OFC	10 [a]	0.5 [c]
Sulfamethoxazole	SMX	6.6 [b]	16 [c]
Trimethoprim	TMP	928 [b]	0.5 [c]
Sulfapyridine	SPD	0.05 [a]	0.05 [b]
Norfloxacin	NFC	120 [b]	0.5 [c]
Sulfadiazine	SDZ	11 [a]	0.05 [b]

[a] Data obtained from Vestel *et al.* [7]
[b] Data obtained from AMR Industry Alliance Secretariat (IFPMA) [6]
[c] Data obtained from Bengtsson-Palme and Larsson [5]

Table S9. Antibiotic concentrations measured in Jewett Creek, Philipps Creek, and South Fork Zumbro River (Aug 2023 – Aug 2024). ND: antibiotic concentration below the method detection limit. <LOQ: concentration below limit of quantification. SMX: sulfamethoxazole, SPD: sufapyridine, SDZ: sulfadiazine, OFC: ofloxacin, CFC: ciprofloxacin, NFC: norfloxacin, TMP: trimethoprim, AZMC: azithromycin, CRMC: clarithromycin. Concentrations are reported in ng L^{-1} ± standard deviation.

Sample ID	Site	SMX	SPD	SDZ	OFC	CFC	NFC	TMP	AZMC	CRMC
JC-2023-A	UPS	ND	ND	ND	ND	ND	42.30 ± 0.10	ND	ND	ND
	DWW	ND	20.60 ± 3.80	29.10 ± 2.60	3.20 ± 0.50	33.10 ± 4.60	62.20 ± 1.60	ND	ND	ND
	DWS1	ND	14.10 ± 5.70	19.00 ± 3.70	2.10 ± 0.30	28.70 ± 4.00	63.50 ± 11.70	2.20 ± 0.30	ND	ND
	DWS2	ND	13.90 ± 0.40	17.50 ± 1.40	0.70 ± 0.01	22.20 ± 0.20	67.70 ± 7.80	1.60 ± 0.10	ND	ND
	DWS3	ND	9.00 ± 0.80	19.50 ± 5.00	0.50 ± 0.20	13.20 ± 3.00	63.80 ± 3.10	3.30 ± 0.20	ND	ND
JC-2023-B	UPS	ND	ND	ND	ND	ND	ND	ND	ND	ND
	DWW	37.30 ± 0.60	51.60 ± 5.40	53.60 ± 2.00	1.30 ± 0.10	10.60 ± 2.40	ND	ND	ND	ND
	DWS1	1.80 ± 0.30	1.10 ± 0.10	1.60 ± 0.10	ND	ND	ND	ND	ND	ND
	DWS2	2.10 ± 0.60	1.20 ± 0.10	1.70 ± 0.30	ND	ND	ND	ND	ND	ND
	DWS3	1.00 ± 0.70	1.20 ± 1.80	2.20 ± 1.00	0.10 ± 0.20	ND	ND	ND	ND	ND
JC-2023-C	UPS	ND	ND	ND	ND	ND	ND	ND	ND	ND
	DWW	110.50 ± 2.60	56.20 ± 8.90	73.70 ± 1.00	0.20 ± 0.10	6.60 ± 0.10	ND	1.10 ± 0.10	ND	ND
	DWS1	21.10 ± 10.40	14.80 ± 11.70	16.10 ± 12.70	0.70 ± 0.30	4.70 ± 2.50	ND	1.30 ± 0.10	ND	ND
	DWS2	11.10 ± 0.30	10.80 ± 1.40	13.30 ± 2.70	0.60 ± 0.40	4.40 ± 0.10	ND	0.50 ± 0.20	ND	ND
	DWS3	1.60 ± 0.50	1.90 ± 1.50	6.70 ± 1.00	0.10 ± 0.10	2.20 ± 0.80	ND	1.90 ± 0.10	ND	ND
JC-2023-D	UPS	ND	ND	ND	ND	ND	ND	ND	ND	ND
	DWW	63.20 ± 2.10	29.40 ± 0.80	30.20 ± 5.80	ND	11.30 ± 0.10	ND	ND	ND	ND
	DWS1	37.10 ± 0.10	11.30 ± 0.20	14.40 ± 3.80	1.20 ± 0.30	9.20 ± 0.03	ND	ND	ND	ND
	DWS2	9.10 ± 0.30	10.20 ± 2.70	11.00 ± 1.20	3.10 ± 0.40	9.10 ± 0.10	ND	ND	ND	ND
	DWS3	4.60 ± 0.60	5.60 ± 0.70	9.20 ± 1.00	1.50 ± 0.00	2.20 ± 0.20	ND	ND	ND	ND
JC-2024-A	UPS	ND	ND	ND	ND	ND	ND	6.48 ± 2.06	ND	ND
	DWW	34.99 ± 0.10	ND	ND	3.14 ± 1.12	ND	ND	4.99 ± 0.03	ND	ND
	DWS1	ND	ND	ND	2.48 ± 0.38	ND	ND	4.90 ± 0.00	ND	ND
	DWS2	ND	ND	ND	2.83 ± 0.97	ND	ND	4.69 ± 0.09	ND	ND
	DWS3	ND	ND	ND	3.69 ± 1.82	ND	ND	4.51 ± 0.39	ND	ND
JC-2024-B	UPS	ND	ND	ND	ND	ND	ND	ND	ND	ND
	DWW	19.03 ± 0.80	43.88 ± 3.04	ND	2.79 ± 0.17	ND	ND	2.19 ± 0.38	ND	ND
	DWS1	ND	ND	ND	ND	ND	ND	5.40 ± 0.43	ND	ND
	DWS2	ND	ND	ND	ND	ND	ND	6.21 ± 0.47	ND	ND
	DWS3	ND	ND	ND	ND	ND	ND	14.65 ± 2.20	ND	ND
JC-2024-C	UPS	ND	ND	ND	ND	ND	ND	ND	ND	ND
	DWW	ND	ND	ND	ND	ND	ND	ND	ND	ND
	DWS1	ND	ND	ND	ND	ND	ND	ND	ND	ND
	DWS2	ND	ND	ND	ND	ND	ND	ND	ND	ND
	DWS3	ND	ND	ND	ND	ND	ND	ND	ND	ND
PC-2023-A	UPS	ND	ND	ND	ND	ND	ND	ND	ND	ND
	EFF	ND	104.00 ± 3.90	159.00 ± 17.60	217.10 ± 20.80	280.90 ± 0.60	72.20 ± 5.90	169.40 ± 10.30	ND	ND
	DWS1	ND	43.90 ± 2.50	ND	46.70 ± 1.10	69.60 ± 2.40	ND	19.90 ± 0.40	ND	ND
	DWS2	ND	41.20 ± 1.30	ND	41.90 ± 1.50	48.60 ± 2.70	ND	22.80 ± 2.60	ND	ND
	DWS3	ND	58.50 ± 5.30	ND	61.40 ± 1.90	131.50 ± 11.90	ND	33.50 ± 6.40	ND	ND
PC-2023-B	UPS	ND	ND	ND	ND	ND	ND	ND	ND	ND

Sample ID	Site	SMX	SPD	SDZ	OFC	CFC	NFC	TMP	AZMC	CRMC
	EFF	62.30 ± 2.70	12.40 ± 0.20	18.40 ± 4.20	203.80 ± 24.70	564.70 ± 60.60	ND	120.80 ± 17.20	4.60 ± 0.00	ND
	DWS1	77.00 ± 6.10	14.00 ± 1.20	18.30 ± 0.90	98.20 ± 48.50	58.40 ± 18.00	ND	131.80 ± 2.80	2.80 ± 0.10	ND
	DWS2	79.70 ± 0.30	16.20 ± 1.50	12.70 ± 2.90	52.10 ± 0.10	52.00 ± 1.00	ND	32.90 ± 2.60	1.00 ± 0.20	ND
	DWS3	34.70 ± 1.00	3.40 ± 4.90	ND	30.80 ± 1.00	45.20 ± 1.00	ND	40.50 ± 1.00	0.70 ± 0.10	ND
PC-2023-C	UPS	ND	ND	ND	ND	ND	ND	ND	ND	ND
	EFF	104.30 ± 3.50	141.50 ± 10.80	102.90 ± 24.30	343.50 ± 228.70	198.70 ± 97.80	ND	74.40 ± 8.90	ND	ND
	DWS1	64.70 ± 14.40	113.40 ± 11.00	89.90 ± 9.20	295.60 ± 4.50	216.80 ± 4.10	ND	66.50 ± 7.70	ND	ND
	DWS2	106.20 ± 7.10	46.60 ± 4.50	54.30 ± 11.90	341.20 ± 99.00	156.00 ± 40.90	ND	32.00 ± 4.80	ND	ND
	DWS3	16.10 ± 1.30	6.30 ± 1.80	8.70 ± 4.50	155.90 ± 32.30	62.90 ± 9.50	ND	25.30 ± 0.30	ND	ND
PC-2023-D	UPS	ND	ND	ND	ND	ND	ND	ND	ND	ND
	EFF	75.60 ± 14.30	323.00 ± 106.50	58.40 ± 14.00	648.20 ± 470.30	428.90 ± 203.10	ND	98.50 ± 43.90	ND	ND
	DWS1	43.90 ± 10.50	109.80 ± 52.40	17.10 ± 4.90	143.70 ± 19.80	331.20 ± 40.20	ND	27.10 ± 9.70	ND	ND
	DWS2	39.00 ± 7.20	17.50 ± 2.70	13.70 ± 1.40	212.20 ± 32.90	112.30 ± 23.20	ND	11.80 ± 0.90	ND	ND
	DWS3	34.90 ± 6.50	18.60 ± 1.40	16.60 ± 1.80	132.40 ± 9.20	68.00 ± 1.80	ND	11.20 ± 2.20	ND	ND
PC-2024-A[1]	UPS	ND	ND	ND	ND	ND	ND	ND	ND	ND
	EFF	662.03 ± 10.18	230.77 ± 5.47	ND	111.29 ± 8.59	35.83 ± 1.61	ND	45.20 ± 5.61	ND	ND
	DWS1	111.81 ± 0.83	24.97 ± 1.02	ND	19.50 ± 3.41	2.39 ± 0.44	ND	13.38 ± 0.58	ND	ND
	DWS2	76.61 ± 1.28	31.50 ± 2.87	ND	10.35 ± 0.51	1.71 ± 0.08	ND	14.69 ± 0.00	ND	ND
	DWS3	53.61 ± 6.26	16.80 ± 1.57	ND	9.86 ± 1.16	0.99 ± 0.35	ND	11.40 ± 0.94	ND	ND
PC-2024-B	UPS	ND	ND	ND	ND	ND	ND	ND	ND	ND
	EFF	396.85 ± 1.76	250.50 ± 10.74	ND	130.42 ± 14.97	69.28 ± 0.30	ND	78.78 ± 8.85	ND	ND
	DWS1	54.98 ± 2.52	8.66 ± 1.17	ND	13.23 ± 0.76	ND	ND	9.28 ± 0.86	ND	ND
	DWS2	28.19 ± 6.00	12.05 ± 0.12	ND	7.21 ± 0.27	ND	ND	10.12 ± 2.34	ND	ND
	DWS3	7.34 ± 1.91	9.85 ± 0.04	ND	7.62 ± 1.27	ND	ND	12.70 ± 0.35	ND	ND
PC-2024-C	UPS	ND	ND	ND	ND	ND	ND	ND	ND	ND
	EFF	556.60 ± 26.12	103.15 ± 7.46	ND	119.25 ± 38.02	159.03 ± 38.06	ND	45.01 ± 2.71	500.31 ± 6.47	11.00 ± 0.81
	DWS1	167.55 ± 12.62	39.65 ± 7.76	ND	9.56 ± 1.00	18.23 ± 10.03	ND	23.69 ± 3.09	ND	2.80 ± 0.01
	DWS2	104.91 ± 7.91	24.11 ± 1.00	ND	5.22 ± 1.00	4.47 ± 1.00	ND	27.09 ± 1.14	ND	2.32 ± 0.19
	DWS3	104.78 ± 4.10	14.85 ± 4.76	ND	3.94 ± 0.33	2.26 ± 0.11	ND	6.82 ± 0.41	ND	3.50 ± 0.40
ZR-2023-A	UPS	ND	ND	ND	ND	ND	63.20 ± 11.30	ND	ND	ND
	DWW	221.60 ± 24.40	24.00 ± 1.90	19.80 ± 1.00	257.90 ± 25.80	22.60 ± 0.40	66.80 ± 5.10	107.00 ± 12.40	2.30 ± 0.10	2.00 ± 0.01
	DWS1	17.50 ± 2.70	8.60 ± 1.10	ND	45.40 ± 19.10	6.80 ± 1.80	57.90 ± 13.80	31.50 ± 0.50	ND	ND
	DWS2	31.60 ± 3.70	13.60 ± 0.10	ND	90.40 ± 4.30	7.40 ± 0.30	71.90 ± 0.01	55.50 ± 7.80	0.90 ± 0.01	0.86 ± 0.01
	DWS3	21.00 ± 4.60	9.70 ± 4.90	ND	12.80 ± 2.40	2.60 ± 3.70	43.60 ± 7.00	5.30 ± 4.10	0.70 ± 0.20	0.68 ± 0.30
ZR-2023-B	UPS	ND	ND	ND	ND	ND	92.50 ± 15.90	ND	ND	ND
	DWW	ND	1.00 ± 0.10	ND	7.70 ± 1.10	ND	43.70 ± 0.40	8.40 ± 2.10	ND	ND
	DWS1	2.00 ± 0.90	21.70 ± 3.00	17.10 ± 2.40	14.30 ± 0.70	5.60 ± 7.90	64.20 ± 2.80	75.80 ± 10.20	2.30 ± 1.00	2.30 ± 0.90
	DWS2	1.90 ± 0.40	13.70 ± 1.10	15.60 ± 0.20	14.60 ± 2.60	3.10 ± 4.30	71.00 ± 8.10	70.20 ± 7.50	2.10 ± 0.10	2.30 ± 0.20
	DWS3	1.80 ± 0.20	13.40 ± 1.20	10.80 ± 0.80	13.00 ± 1.80	ND	62.30 ± 0.70	41.90 ± 5.50	1.50 ± 0.60	1.70 ± 0.50
ZR-2023-C	UPS	ND	ND	ND	ND	ND	104.90 ± 1.00	4.20 ± 1.00	ND	ND
	DWW	10.20 ± 2.00	22.80 ± 0.30	133.00 ± 12.40	147.70 ± 14.80	221.80 ± 77.20	48.80 ± 3.70	444.10 ± 77.50	ND	ND
	DWS1	5.80 ± 1.20	30.70 ± 5.00	49.20 ± 6.80	36.90 ± 6.50	77.90 ± 15.60	101.70 ± 1.00	193.50 ± 48.20	ND	ND
	DWS2	5.60 ± 0.40	15.90 ± 1.60	19.00 ± 3.70	63.30 ± 3.00	43.00 ± 0.20	104.20 ± 16.60	124.80 ± 12.00	ND	ND
	DWS3	4.30 ± 0.20	16.40 ± 1.30	24.70 ± 3.40	21.50 ± 3.00	22.90 ± 2.60	37.00 ± 0.70	152.60 ± 8.40	ND	ND
ZR-2024-A	UPS	ND	ND	ND	ND	ND	ND	ND	ND	ND
	DWW	26.02 ± 0.82	8.86 ± 0.15	ND	175.30 ± 22.97	29.53 ± 4.04	ND	520.50 ± 31.79	344.46 ± 29.70	27.98 ± 3.65
	DWS1	26.02 ± 1.26	6.52 ± 2.60	ND	3.93 ± 0.60	ND	ND	10.11 ± 0.90	3.91 ± 1.00	0.50 ± 0.04

Sample ID	Site	SMX	SPD	SDZ	OFC	CFC	NFC	TMP	AZMC	CRMC
	DWS2	40.51 ± 5.23	13.53 ± 1.57	ND	12.12 ± 3.47	ND	ND	22.60 ± 5.10	7.46 ± 1.00	1.27 ± 0.10
	DWS3	54.02 ± 1.00	30.38 ± 1.00	ND	12.24 ± 1.00	ND	ND	20.76 ± 1.00	4.63 ± 1.00	1.27 ± 0.05
ZR-2024-B	UPS	ND	ND	ND	ND	ND	ND	ND	ND	ND
	DWW	58.98 ± 15.39	12.65 ± 2.53	ND	823.19 ± 81.97	35.32 ± 3.83	ND	402.03 ± 10.85	232.53 ± 4.93	35.17 ± 2.26
	DWS1	56.78 ± 10.39	16.67 ± 6.21	ND	35.19 ± 3.59	12.26 ± 3.19	ND	45.23 ± 5.90	7.30 ± 1.00	6.19 ± 5.06
	DWS2	53.09 ± 1.08	18.26 ± 1.02	ND	26.01 ± 1.33	4.74 ± 2.32	ND	37.41 ± 2.50	6.63 ± 1.00	2.24 ± 0.15
	DWS3	30.15 ± 4.79	61.72 ± 11.28	ND	28.19 ± 10.61	2.77 ± 0.96	ND	30.23 ± 1.24	7.31 ± 1.00	4.76 ± 1.44
ZR-2024-C	UPS	ND	ND	ND	ND	ND	ND	ND	ND	ND
	DWW	70.07 ± 1.00	52.86 ± 1.00	ND	300.52 ± 5.00	129.09 ± 1.00	ND	170.53 ± 1.00	216.76 ± 1.00	56.42 ± 1.00
	DWS1	44.58 ± 0.21	45.41 ± 0.64	ND	53.56 ± 8.45	10.78 ± 0.76	ND	59.39 ± 0.24	97.76 ± 3.61	20.30 ± 4.15
	DWS2	26.48 ± 0.69	31.17 ± 0.18	ND	10.20 ± 0.06	4.27 ± 0.74	ND	21.82 ± 0.33	16.58 ± 0.70	2.77 ± 0.22
	DWS3	25.85 ± 1.00	26.02 ± 1.00	ND	8.27 ± 1.00	1.95 ± 1.00	ND	10.23 ± 1.00	10.70 ± 1.00	3.93 ± 1.00

[1] Amoxicillin (AMX) was detected exclusively in PC-2024-A, with concentrations measured at 72.22 ± 2.82 ng/L in DWS1, 35.27 ± 1.13 ng/L in DWS2, and 14.70 ± 2.61 ng/L in DWS3.

Table S10. Absolute recovery (%) of individual antibiotics in spiked samples from each sampling event.

Sample ID/ Collection date	Site	SMX	SPD	SDZ	OFC	CFC	NFC	TMP	AZMC	CRMC
JC-2023-A	DWW	70	85	94	94	85	52	149	12	13
08/07/2023	DWS2	97	108	140	93	72	36	154	17	18
JC-2023-B	DWW	27	49	57	25	62	NC	23	25	NC
09/06/2023	DWS1	50	55	35	59	30	NC	10	10	NC
JC-2023-C	DWW	12	26	22	50	45	NC	25	18	NC
09/22/2023	DWS1	33	56	61	45	52	NC	44	50	NC
JC-2023-D	DWW	38	74	80	20	50	25	10	20	7
10/11/2023	DWS1	27	109	98	80	115	90	53	22	21
JC-2024-A	UPS	25	10	15	8	15	10	44	NC	NC
05/14/2024	DWW	21	8	42	40	124	27	40	NC	NC
	DWS1	21	8	42	31	10	15	40	NC	NC
	DWS2	11	11	35	31	24	15	42	NC	NC
	DWS3	33	96	26	43	94	16	38	NC	NC
JC-2024-B	UPS	43	102	113	51	21	149	97	NC	54
06/05/2024	DWW	52	143	135	89	7	46	99	NC	22
	DWS1	29	102	138	57	7	47	96	NC	53
	DWS2	50	113	125	77	6	10	100	NC	50
	DWS3	27	114	106	27	15	42	119	NC	13
JC-2024-C	UPS	37	92	88	95	26	24	95	29	8
07/02/2024	DWW	30	69	56	50	55	4	74	33	6
	DWS1	51	70	25	40	34	49	66	27	5
	DWS2	38	49	42	79	49	28	73	44	7
	DWS3	26	42	36	54	25	113	51	12	3
PC-2023-A	EFF	60	14	17	59	166	124	40	11	15
08/10/2023	DWS2	110	101	45	101	189	117	74	15	18
PC-2023-B	EFF	55	30	55	33	46	NC	49	14	NC
09/12/2023	DWS1	40	35	40	34	90	NC	26	24	NC
PC-2023-C	EFF	92	37	59	61	61	35	41	8	7
09/28/2023	DWS1	50	17	20	60	87	87	62	9	9
PC-2023-D	EFF	50	21	12	50	55	35	37	3	3
10/18/2023	DWS1	44	20	27	129	37	47	45	5	5
PC-2024-A	UPS	31	106	95	3	24	122	110	NC	38
05/29/2024	EFF	74	75	114	72	110	64	164	NC	18
	DWS1	47	98	98	28	106	2	100	NC	53
	DWS2	61	71	66	52	18	4	97	NC	31
	DWS3	78	92	60	41	19	30	98	NC	48
PC-2024-B	UPS	35	93	100	32	24	66	94	NC	1

Sample ID/ Collection date	Site	SMX	SPD	SDZ	OFC	CFC	NFC	TMP	AZMC	CRMC
06/11/2024	EFF	39	93	68	74	60	66	106	NC	8
	DWS1	23	59	37	48	35	47	95	NC	1
	DWS2	37	72	28	74	29	160	119	NC	1
	DWS3	98	129	68	85	36	79	109	NC	1
PC-2024-C	UPS	62	96	67	75	27	146	105	NC	4
07/09/2024	EFF	69	75	47	41	18	29	143	35	6
	DWS1	24	32	34	98	46	104	75	NC	4
	DWS2	31	90	35	32	17	50	39	NC	6
	DWS3	23	47	31	41	19	2	90	NC	3
ZR-2023-A	DWW	22	30	10	126	78	99	11	7	7
06/29/2023	DWS3	55	45	36	94	138	158	14	15	15
ZR-2023-B	DWW	70	61	65	88	130	113	145	36	38
07/25/2023	DWS3	20	29	36	83	147	111	162	10	11
ZR-2023-C	DWW	10	5	17	48	98	123	73	14	15
08/23/2023	DWS2	10	21	20	50	90	102	131	12	45
ZR-2024-A	UPS	33	51	53	13	24	21	117	25	30
07/18/2024	DWW	50	-2	25	140	48	186	61	42	12
	DWS1	50	55	63	39	29	24	94	69	38
	DWS2	70	75	79	37	32	3	107	36	35
	DWS3	70	55	67	43	66	174	102	58	41
ZR-2024-B	UPS	27	33	28	50	34	4	91	70	14
07/29/2024	DWW	30	1	25	32	56	15	73	111	6
	DWS1	25	82	30	83	28	52	103	37	26
	DWS2	34	76	31	32	18	52	39	41	31
	DWS3	17	41	47	44	21	53	43	37	30
ZR-2024-C	UPS	40	75	74	40	21	57	44	37	26
08/05/2024	DWW	53	22	6	72	23	66	62	119	12
	DWS1	43	52	28	79	33	61	51	68	21
	DWS2	36	77	47	17	17	33	40	31	39
	DWS3	56	83	25	127	52	106	86	91	39

NC: Calculation was not possible due to poor internal standard performance in the spiked sample.
In 2023, only two samples were divided into triplicates for the spiking calculation of absolute recovery, owing to limited sample volume. This limitation was addressed in 2024, with all samples from each event being split in triplicate, including the spiked sample.

Table S11. Apparent in-stream attenuation rate constants in units of m^{-1} (k_d) and s^{-1} (k_t), 95% confidence interval, model fit (R^2), and number of points for the regression model of measured antibiotics on each sampling event.

Sample identification	Parameters	CFC	NFC	OFC	SDZ	SPD	SMX	TMP	AZMC	CRMC	AMX
JC-2023-A	k_d (m^{-1})	2.26E-04	-8.42E-08	3.99E-04	4.99E-05	1.83E-04	-----[a]	-----[a]	-----[a]	-----[a]	-----[a]
	k_d 95% CI (m^{-1})	2.05E-04	6.45E-05	9.84E-04	3.73E-04	2.21E-04	-----[a]	-----[a]	-----[a]	-----[a]	-----[a]
	k_t (s^{-1})	9.04E-05	-3.37E-08	1.59E-04	2.00E-05	7.34E-05	-----[a]	-----[a]	-----[a]	-----[a]	-----[a]
	k_t 95% CI (s^{-1})	8.22E-05	2.58E-05	3.94E-04	1.49E-04	8.84E-05	-----[a]	-----[a]	-----[a]	-----[a]	-----[a]
	R^2	0.9180	0.0000	0.6029	0.1421	0.8645	-----[a]	-----[a]	-----[a]	-----[a]	-----[a]
	Points	4	4	4	4	4	-----[a]	-----[a]	-----[a]	-----[a]	-----[a]
JC-2023-B	k_d (m^{-1})	-----[a]	-----[a]	-----[a]	4.31E-04	5.49E-04	6.26E-04	-----[a]	-----[a]	-----[a]	-----[a]
	k_d 95% CI (m^{-1})	-----[a]	-----[a]	-----[a]	2.71E-03	2.92E-03	2.15E-03	-----[a]	-----[a]	-----[a]	-----[a]
	k_t (s^{-1})	-----[a]	-----[a]	-----[a]	1.72E-04	2.20E-04	2.50E-04	-----[a]	-----[a]	-----[a]	-----[a]
	k_t 95% CI (s^{-1})	-----[a]	-----[a]	-----[a]	1.08E-03	1.17E-03	8.60E-04	-----[a]	-----[a]	-----[a]	-----[a]
	R^2	-----[a]	-----[a]	1	0.1898	0.2468	0.4395	-----[a]	-----[a]	-----[a]	-----[a]
	Points	1	-----[a]	2	4	4	4	-----[a]	-----[a]	-----[a]	-----[a]
JC-2023-C	k_d (m^{-1})	2.63E-04	-----[a]	3.61E-04	4.62E-04	7.63E-04	9.39E-04	-1.98E-04	-----[a]	-----[a]	-----[a]
	k_d 95% CI (m^{-1})	1.68E-04	-----[a]	1.22E-03	1.11E-03	8.48E-04	1.21E-03	7.94E-04	-----[a]	-----[a]	-----[a]
	k_t (s^{-1})	1.08E-04	-----[a]	1.48E-04	1.89E-04	3.12E-04	3.84E-04	-8.09E-05	-----[a]	-----[a]	-----[a]
	k_t 95% CI (s^{-1})	6.86E-05	-----[a]	4.99E-04	4.55E-04	3.47E-04	4.97E-04	3.25E-04	-----[a]	-----[a]	-----[a]
	R^2	0.9579	-----[a]	0.4491	0.6156	0.8823	0.8469	0.3643	-----[a]	-----[a]	-----[a]
	Points	4	-----[a]	4	4	4	4	4	-----[a]	-----[a]	-----[a]
JC-2023-D	k_d (m^{-1})	4.39E-04	-----[a]	-----[a]	2.19E-04	3.35E-04	5.81E-04	-----[a]	-----[a]	-----[a]	-----[a]
	k_d 95% CI (m^{-1})	7.88E-05	-----[a]	-----[a]	6.45E-04	6.64E-04	1.23E-03	-----[a]	-----[a]	-----[a]	-----[a]
	k_t (s^{-1})	1.76E-04	-----[a]	-----[a]	8.77E-05	1.34E-04	2.32E-04	-----[a]	-----[a]	-----[a]	-----[a]
	k_t 95% CI (s^{-1})	3.15E-05	-----[a]	-----[a]	2.58E-04	2.66E-04	4.92E-04	-----[a]	-----[a]	-----[a]	-----[a]
	R^2	0.9965	-----[a]	-----[a]	0.5170	0.7022	0.6739	-----[a]	-----[a]	-----[a]	-----[a]
	Points	4	-----[a]	-----[a]	4	4	4	-----[a]	-----[a]	-----[a]	-----[a]
JC-2024-A	k_d (m^{-1})	-----[a]	-----[a]	-6.98E-05	-----[a]	-----[a]	-----[a]	2.34E-05	-----[a]	-----[a]	-----[a]
	k_d 95% CI (m^{-1})	-----[a]	-----[a]	2.09E-04	-----[a]	-----[a]	-----[a]	3.72E-05	-----[a]	-----[a]	-----[a]
	k_t (s^{-1})	-----[a]	-----[a]	-2.38E-05	-----[a]	-----[a]	-----[a]	7.95E-06	-----[a]	-----[a]	-----[a]
	k_t 95% CI (s^{-1})	-----[a]	-----[a]	7.12E-05	-----[a]	-----[a]	-----[a]	1.27E-05	-----[a]	-----[a]	-----[a]
	R^2	-----[a]	-----[a]	0.5084	-----[a]	-----[a]	-----[a]	0.7845	-----[a]	-----[a]	-----[a]
	Points	-----[a]	-----[a]	4	-----[a]	-----[a]	1	4	-----[a]	-----[a]	-----[a]
JC-2024-B	k_d (m^{-1})	-----[a]	-----[a]	-----[a]	-----[a]	-----[a]	-----[a]	-4.09E-04	-----[a]	-----[a]	-----[a]
	k_d 95% CI (m^{-1})	-----[a]	-----[a]	-----[a]	-----[a]	-----[a]	-----[a]	5.94E-04	-----[a]	-----[a]	-----[a]
	k_t (s^{-1})	-----[a]	-----[a]	-----[a]	-----[a]	-----[a]	-----[a]	-1.39E-04	-----[a]	-----[a]	-----[a]
	k_t 95% CI (s^{-1})	-----[a]	-----[a]	-----[a]	-----[a]	-----[a]	-----[a]	2.02E-04	-----[a]	-----[a]	-----[a]
	R^2	-----[a]	-----[a]	-----[a]	-----[a]	-----[a]	-----[a]	0.8148	-----[a]	-----[a]	-----[a]
	Points	-----[a]	-----[a]	1	-----[a]	1	1	4	-----[a]	-----[a]	-----[a]
PC-2023-A	k_d (m^{-1})	-2.03E-03	-----[a]	-8.23E-04	-----[a]	-8.08E-04	-----[a]	-1.19E-03	-----[a]	-----[a]	-----[a]
	k_d 95% CI (m^{-1})	1.25E-02	-----[a]	4.01E-03	-----[a]	2.71E-03	-----[a]	2.56E-03	-----[a]	-----[a]	-----[a]
	k_t (s^{-1})	-2.03E-04	-----[a]	-8.23E-05	-----[a]	-8.08E-05	-----[a]	-1.19E-04	-----[a]	-----[a]	-----[a]
	k_t 95% CI (s^{-1})	1.25E-03	-----[a]	4.01E-04	-----[a]	2.71E-04	-----[a]	2.56E-04	-----[a]	-----[a]	-----[a]

Sample identification	Parameters	CFC	NFC	OFC	SDZ	SPD	SMX	TMP	AZMC	CRMC	AMX
	R^2	0.8095	-----[a]	0.8717	-----[a]	0.9348	-----[a]	0.9723	-----[a]	-----[a]	-----[a]
	Points	3	-----[a]	3	-----[a]	3	-----[a]	3	-----[a]	-----[a]	-----[a]
PC-2023-B	k_d (m^{-1})	5.33E-04	-----[a]	2.29E-03	-----[a]	3.80E-03	2.09E-03	1.52E-03	2.44E-03	-----[a]	-----[a]
	k_d 95% CI (m^{-1})	2.72E-03	-----[a]	1.55E-02	-----[a]	8.40E-03	3.31E-03	3.79E-02	2.67E-02	-----[a]	-----[a]
	k_t (s^{-1})	5.29E-05	-----[a]	2.27E-04	-----[a]	3.77E-04	2.07E-04	1.51E-04	2.42E-04	-----[a]	-----[a]
	k_t 95% CI (s^{-1})	2.70E-04	-----[a]	1.54E-03	-----[a]	8.33E-04	3.28E-04	3.76E-03	2.65E-03	-----[a]	-----[a]
	R^2	0.8610	-----[a]	0.7785	-----[a]	0.9707	0.9847	0.2065	0.5756	-----[a]	-----[a]
	Points	3	-----[a]	3	2	3	3	3	3	-----[a]	-----[a]
PC-2023-C	k_d (m^{-1})	2.83E-03	-----[a]	1.80E-03	5.46E-03	6.47E-03	4.12E-03	1.69E-03	-----[a]	-----[a]	-----[a]
	k_d 95% CI (m^{-1})	6.25E-03	-----[a]	6.10E-03	8.31E-03	1.82E-02	1.87E-02	1.90E-02	-----[a]	-----[a]	-----[a]
	k_t (s^{-1})	4.37E-04	-----[a]	2.79E-04	8.45E-04	1.00E-03	6.38E-04	2.61E-04	-----[a]	-----[a]	-----[a]
	k_t 95% CI (s^{-1})	9.67E-04	-----[a]	9.44E-04	1.29E-03	2.81E-03	2.90E-03	2.94E-03	-----[a]	-----[a]	-----[a]
	R^2	0.9706	-----[a]	0.9338	0.9859	0.9534	0.8866	0.5607	-----[a]	-----[a]	-----[a]
	Points	3	-----[a]	3	3	3	3	3	-----[a]	-----[a]	-----[a]
PC-2023-D	k_d (m^{-1})	2.89E-03	-----[a]	6.36E-04	-1.66E-04	2.57E-03	4.61E-04	1.37E-03	-----[a]	-----[a]	-----[a]
	k_d 95% CI (m^{-1})	2.76E-02	-----[a]	1.18E-02	6.51E-03	4.96E-02	2.86E-03	2.22E-02	-----[a]	-----[a]	-----[a]
	k_t (s^{-1})	2.89E-04	-----[a]	6.36E-05	-1.66E-05	2.57E-04	4.61E-05	1.37E-04	-----[a]	-----[a]	-----[a]
	k_t 95% CI (s^{-1})	2.76E-03	-----[a]	1.18E-03	6.51E-04	4.96E-03	2.86E-04	2.22E-03	-----[a]	-----[a]	-----[a]
	R^2	0.6389	-----[a]	0.3183	0.0946	0.3019	0.8073	0.3794	-----[a]	-----[a]	-----[a]
	Points	3	-----[a]	3	3	3	3	3	-----[a]	-----[a]	-----[a]
PC-2024-A	k_d (m^{-1})	1.89E-03	-----[a]	1.06E-03	-----[a]	1.27E-03	1.48E-03	5.14E-04	-----[a]	-----[a]	3.32E-03
	k_d 95% CI (m^{-1})	7.45E-03	-----[a]	1.69E-02	-----[a]	8.06E-03	9.15E-03	3.24E-03	-----[a]	-----[a]	1.68E-02
	k_t (s^{-1})	5.92E-04	-----[a]	3.33E-04	-----[a]	3.99E-04	4.63E-04	1.61E-04	-----[a]	-----[a]	1.04E-03
	k_t 95% CI (s^{-1})	2.33E-03	-----[a]	5.28E-03	-----[a]	2.52E-03	2.86E-03	1.01E-03	-----[a]	-----[a]	5.25E-03
	R^2	0.9124	-----[a]	0.3907	-----[a]	0.8016	0.8085	0.8028	-----[a]	-----[a]	0.8632
	Points	3	-----[a]	3	-----[a]	3	3	3	-----[a]	-----[a]	3
PC-2024-B	k_d (m^{-1})	-----[a]	-----[a]	7.57E-04	-----[a]	2.95E-05	4.45E-03	-7.13E-04	-----[a]	-----[a]	-----[a]
	k_d 95% CI (m^{-1})	-----[a]	-----[a]	1.65E-02	-----[a]	9.47E-03	1.41E-02	1.67E-03	-----[a]	-----[a]	-----[a]
	k_t (s^{-1})	-----[a]	-----[a]	3.26E-04	-----[a]	1.27E-05	1.92E-03	-3.07E-04	-----[a]	-----[a]	-----[a]
	k_t 95% CI (s^{-1})	-----[a]	-----[a]	7.11E-03	-----[a]	4.08E-03	6.08E-03	7.21E-04	-----[a]	-----[a]	-----[a]
	R^2	-----[a]	-----[a]	0.2538	-----[a]	0.0016	0.9414	0.9671	-----[a]	-----[a]	-----[a]
	Points	-----[a]	-----[a]	3	-----[a]	3	3	3	-----[a]	-----[a]	-----[a]
PC-2024-C	k_d (m^{-1})	3.84E-03	-----[a]	1.62E-03	-----[a]	1.99E-03	6.97E-04	3.35E-03	-----[a]	-7.80E-04	-----[a]
	k_d 95% CI (m^{-1})	3.59E-02	-----[a]	1.55E-02	-----[a]	1.20E-02	1.26E-02	7.56E-03	-----[a]	6.24E-03	-----[a]
	k_t (s^{-1})	1.20E-03	-----[a]	5.08E-04	-----[a]	6.22E-04	2.18E-04	1.05E-03	-----[a]	-2.44E-04	-----[a]
	k_t 95% CI (s^{-1})	1.12E-02	-----[a]	4.84E-03	-----[a]	3.75E-03	3.94E-03	2.37E-03	-----[a]	1.95E-03	-----[a]
	R^2	0.6494	-----[a]	0.6395	-----[a]	0.8157	0.3313	0.9695	-----[a]	0.7163	-----[a]
	Points	3	-----[a]	3	-----[a]	3	3	3	-----[a]	3	-----[a]
ZR-2023-A	k_d (m^{-1})	7.89E-04	1.89E-04	1.12E-03	-----[a]	2.45E-04	6.27E-04	1.22E-03	4.47E-04	4.06E-04	-----[a]
	k_d 95% CI (m^{-1})	1.19E-03	3.43E-04	1.73E-03	-----[a]	1.18E-03	2.98E-03	1.21E-03	3.74E-03	3.35E-03	-----[a]
	R^2	0.8024	0.7377	0.7946	-----[a]	0.2866	0.2908	0.9048	0.6977	0.7038	-----[a]
	Points	4	4	4	1	4	4	4	3	3	-----[a]
ZR-2023-B	k_d (m^{-1})	-----[a]	-8.15E-05	-1.18E-04	-----[a]	-6.17E-04		-3.05E-04	-----[a]	-----[a]	-----[a]
	k_d 95% CI (m^{-1})	-----[a]	5.91E-04	8.40E-04	-----[a]	3.80E-03		2.96E-03	-----[a]	-----[a]	-----[a]
	R^2	-----[a]	0.1494	0.1536	-----[a]	0.1961		0.0898	-----[a]	-----[a]	-----[a]
	Points	-----[a]	4	4	-----[a]	4		4	-----[a]	-----[a]	-----[a]

Sample identification	Parameters	CFC	NFC	OFC	SDZ	SPD	SMX	TMP	AZMC	CRMC	AMX
ZR-2023-C	k_d (m^{-1})	8.31E-04	2.94E-04	6.65E-04	5.36E-04	1.80E-04	2.92E-04	3.07E-04	-----[a]	-----[a]	-----[a]
	k_d 95% CI (m^{-1})	1.47E-03	1.31E-03	1.45E-03	2.07E-03	7.62E-04	6.49E-04	1.41E-03	-----[a]	-----[a]	-----[a]
	R^2	0.7461	0.3173	0.6602	0.3832	0.3416	0.6529	0.3065	-----[a]	-----[a]	-----[a]
	Points	4	4	4	4	4	4	4	-----[a]	-----[a]	-----[a]
ZR-2024-A	k_d (m^{-1})	-----[a]	-----[a]	5.37E-04	-----[a]	-6.13E-04	-3.24E-04	7.55E-04	1.15E-03	6.83E-04	-----[a]
	k_d 95% CI (m^{-1})	-----[a]	-----[a]	4.60E-03	-----[a]	7.81E-04	4.58E-04	4.79E-03	5.38E-03	4.90E-03	-----[a]
	R^2	-----[a]	-----[a]	0.1118	-----[a]	0.8508	0.8218	0.1869	0.2982	0.1523	-----[a]
	Points	1	-----[a]	4	-----[a]	4	4	4	4	4	-----[a]
ZR-2024-B	k_d (m^{-1})	9.38E-04	-----[a]	9.27E-04	-----[a]	-6.99E-04	3.11E-04	7.64E-04	9.15E-04	5.24E-04	-----[a]
	k_d 95% CI (m^{-1})	1.83E-03	-----[a]	4.19E-03	-----[a]	1.16E-04	1.15E-04	2.81E-03	4.50E-03	3.15E-03	-----[a]
	R^2	0.7075	-----[a]	0.3114	-----[a]	0.9970	0.9853	0.4071	0.2767	0.2039	-----[a]
	Points	4	-----[a]	4	-----[a]	4	4	4	4	4	-----[a]
ZR-2024-C	k_d (m^{-1})	1.43E-03	-----[a]	1.25E-03	-----[a]	2.85E-04	3.50E-04	1.06E-03	1.16E-03	9.35E-04	-----[a]
	k_d 95% CI (m^{-1})	3.40E-03	-----[a]	3.34E-03	-----[a]	4.86E-04	9.72E-04	1.80E-03	2.49E-03	3.21E-03	-----[a]
	R^2	0.6189	-----[a]	0.5652	-----[a]	0.7608	0.5446	0.7647	0.6685	0.4407	-----[a]
	Points	4	-----[a]	4	-----[a]	4	4	4	4	4	-----[a]

[a] indicates that antibiotics were either not detected or found in only one or two samples

Table S12. Mean, standard deviation, minimum, and maximum NPMLE of individual antibiotics in the downstream mixing (DWW) sample for Jewett Creek and South Fork Zumbro River, and in downstream 1 (DWS1) sample in Philipps Creek.

Site	Antibiotic	Number of detected antibiotics	Mean NPMLE	Standard deviation	Minimum NPMLE	Maximum NPMLE
Jewett Creek (Population = 6,541)	SMX	5	3359	2343	1049	7087
	SPD	5	2627	3418	580	8695
	SDZ	4	1313	599	819	2074
	TMP	3	492	493	31	1011
	OFC	5	264	304	6	636
	CFC	4	433	337	186	931
	NFC	1	1750	NA	NA	NA
Philipps Creek (Population = 8,240)	SMX	6	3137	3997	69	9324
	SPD	7	656	855	17	2206
	SDZ	3	77	90	22	181
	TMP	7	415	484	31	1318
	OFC	7	473	383	73	1185
	CFC	6	383	360	71	1014
	AMX	1	4387	NA	NA	NA
	AZMC	1	3	NA	NA	NA
	CRMC	1	156	NA	NA	NA
South Fork Zumbro River (Population = 112,413)	SMX	5	247	170	10	460
	SPD	6	75	95	1	261
	SDZ	2	83	59	41	124
	TMP	6	1219	1680	11	4515
	OFC	6	1062	1025	10	2682
	CFC	5	252	230	47	637
	NFC	3	81	51	45	139
	AZMC	4	1205	1270	5	2988
	CRMC	4	160	125	4	279

NA means only one antibiotic was detected.

Table S13. Risk assessment calculated for all antibiotics measured in this study.

Sample ID	Site	Antibiotic	Concentration (ng L^{-1})	RQ_{ENV}	RQ_{AMR}	RQ_{AMR} (class)	RQ_{ENV} (class)
PC-2023-B	DWS1	SMX	77	0.01	0	< 0.01	< 0.01
PC-2023-B	DWS2	SMX	79.7	0.01	0	< 0.01	< 0.01
PC-2023-B	DWS3	SMX	34.7	0.01	0	< 0.01	< 0.01
PC-2023-C	DWS1	SMX	64.7	0.01	0	< 0.01	< 0.01
PC-2023-C	DWS2	SMX	106.2	0.02	0.01	< 0.01	0.01 - 0.1
PC-2023-C	DWS3	SMX	16.1	0	0	< 0.01	< 0.01
PC-2023-D	DWS1	SMX	43.9	0.01	0	< 0.01	< 0.01
PC-2023-D	DWS2	SMX	39	0.01	0	< 0.01	< 0.01
PC-2023-D	DWS3	SMX	34.9	0.01	0	< 0.01	< 0.01
JC-2023-B	DWW	SMX	37.3	0.01	0	< 0.01	< 0.01
JC-2023-B	DWS1	SMX	1.8	0	0	< 0.01	< 0.01
JC-2023-B	DWS2	SMX	2.1	0	0	< 0.01	< 0.01
JC-2023-B	DWS3	SMX	1	0	0	< 0.01	< 0.01
JC-2023-C	DWW	SMX	110.5	0.02	0.01	< 0.01	0.01 - 0.1
JC-2023-C	DWS1	SMX	21.1	0	0	< 0.01	< 0.01
JC-2023-C	DWS2	SMX	11.1	0	0	< 0.01	< 0.01
JC-2023-C	DWS3	SMX	1.6	0	0	< 0.01	< 0.01
JC-2023-D	DWW	SMX	63.2	0.01	0	< 0.01	< 0.01
JC-2023-D	DWS1	SMX	37.1	0.01	0	< 0.01	< 0.01
JC-2023-D	DWS2	SMX	9.1	0	0	< 0.01	< 0.01
JC-2023-D	DWS3	SMX	4.6	0	0	< 0.01	< 0.01
ZR-2023-A	DWW	SMX	221.6	0.03	0.01	< 0.01	0.01 - 0.1
ZR-2023-A	DWS1	SMX	17.5	0	0	< 0.01	< 0.01
ZR-2023-A	DWS2	SMX	31.6	0	0	< 0.01	< 0.01
ZR-2023-A	DWS3	SMX	21	0	0	< 0.01	< 0.01
ZR-2023-B	DWW	SMX	0	0	0	< 0.01	< 0.01
ZR-2023-B	DWS1	SMX	2	0	0	< 0.01	< 0.01
ZR-2023-B	DWS2	SMX	1.9	0	0	< 0.01	< 0.01
ZR-2023-B	DWS3	SMX	1.8	0	0	< 0.01	< 0.01
ZR-2023-C	DWW	SMX	10.2	0	0	< 0.01	< 0.01
ZR-2023-C	DWS1	SMX	5.8	0	0	< 0.01	< 0.01
ZR-2023-C	DWS2	SMX	5.6	0	0	< 0.01	< 0.01
ZR-2023-C	DWS3	SMX	4.3	0	0	< 0.01	< 0.01
PC-2024-A	DWS1	SMX	111.81	0.02	0.01	< 0.01	0.01 - 0.1
PC-2024-A	DWS2	SMX	76.61	0.01	0	< 0.01	< 0.01
PC-2024-A	DWS3	SMX	53.61	0.01	0	< 0.01	< 0.01
PC-2024-B	DWS1	SMX	54.98	0.01	0	< 0.01	< 0.01
PC-2024-B	DWS2	SMX	28.19	0	0	< 0.01	< 0.01
PC-2024-B	DWS3	SMX	7.34	0	0	< 0.01	< 0.01
PC-2024-C	DWS1	SMX	167.55	0.03	0.01	< 0.01	0.01 - 0.1
PC-2024-C	DWS2	SMX	104.91	0.02	0.01	< 0.01	0.01 - 0.1
PC-2024-C	DWS3	SMX	104.78	0.02	0.01	< 0.01	0.01 - 0.1
JC-2024-A	DWW	SMX	34.99	0.01	0	< 0.01	< 0.01
JC-2024-B	DWW	SMX	19.03	0	0	< 0.01	< 0.01
ZR-2024-A	DWW	SMX	26.02	0	0	< 0.01	< 0.01
ZR-2024-A	DWS1	SMX	26.02	0	0	< 0.01	< 0.01
ZR-2024-A	DWS2	SMX	40.51	0.01	0	< 0.01	< 0.01
ZR-2024-A	DWS3	SMX	54.02	0.01	0	< 0.01	< 0.01
ZR-2024-B	DWW	SMX	58.98	0.01	0	< 0.01	< 0.01
ZR-2024-B	DWS1	SMX	56.78	0.01	0	< 0.01	< 0.01
ZR-2024-B	DWS2	SMX	53.09	0.01	0	< 0.01	< 0.01
ZR-2024-B	DWS3	SMX	30.15	0	0	< 0.01	< 0.01
ZR-2024-C	DWW	SMX	70.07	0.01	0	< 0.01	< 0.01
ZR-2024-C	DWS1	SMX	44.58	0.01	0	< 0.01	< 0.01
ZR-2024-C	DWS2	SMX	26.48	0	0	< 0.01	< 0.01
ZR-2024-C	DWS3	SMX	25.85	0	0	< 0.01	< 0.01
PC-2023-A	DWS1	SPD	43.9	0.88	0.88	0.1 - 1	0.1 - 1
PC-2023-A	DWS2	SPD	41.2	0.82	0.82	0.1 - 1	0.1 - 1

Sample ID	Site	Antibiotic	Concentration (ng L^{-1})	RQ_{ENV}	RQ_{AMR}	RQ_{AMR} (class)	RQ_{ENV} (class)
PC-2023-A	DWS3	SPD	58.5	1.17	1.17	> 1	> 1
PC-2023-B	DWS1	SPD	14	0.28	0.28	0.1 - 1	0.1 - 1
PC-2023-B	DWS2	SPD	16.2	0.32	0.32	0.1 - 1	0.1 - 1
PC-2023-B	DWS3	SPD	3.4	0.07	0.07	0.01 - 0.1	0.01 - 0.1
PC-2023-C	DWS1	SPD	113.4	2.27	2.27	> 1	> 1
PC-2023-C	DWS2	SPD	46.6	0.93	0.93	0.1 - 1	0.1 - 1
PC-2023-C	DWS3	SPD	6.3	0.13	0.13	0.1 - 1	0.1 - 1
PC-2023-D	DWS1	SPD	109.8	2.2	2.2	> 1	> 1
PC-2023-D	DWS2	SPD	17.5	0.35	0.35	0.1 - 1	0.1 - 1
PC-2023-D	DWS3	SPD	18.6	0.37	0.37	0.1 - 1	0.1 - 1
JC-2023-A	DWW	SPD	20.6	0.41	0.41	0.1 - 1	0.1 - 1
JC-2023-A	DWS1	SPD	14.1	0.28	0.28	0.1 - 1	0.1 - 1
JC-2023-A	DWS2	SPD	13.9	0.28	0.28	0.1 - 1	0.1 - 1
JC-2023-A	DWS3	SPD	9	0.18	0.18	0.1 - 1	0.1 - 1
JC-2023-B	DWW	SPD	51.6	1.03	1.03	> 1	> 1
JC-2023-B	DWS1	SPD	1.1	0.02	0.02	0.01 - 0.1	0.01 - 0.1
JC-2023-B	DWS2	SPD	1.2	0.02	0.02	0.01 - 0.1	0.01 - 0.1
JC-2023-B	DWS3	SPD	1.2	0.02	0.02	0.01 - 0.1	0.01 - 0.1
JC-2023-C	DWW	SPD	56.2	1.12	1.12	> 1	> 1
JC-2023-C	DWS1	SPD	14.8	0.3	0.3	0.1 - 1	0.1 - 1
JC-2023-C	DWS2	SPD	10.8	0.22	0.22	0.1 - 1	0.1 - 1
JC-2023-C	DWS3	SPD	1.9	0.04	0.04	0.01 - 0.1	0.01 - 0.1
JC-2023-D	DWW	SPD	29.4	0.59	0.59	0.1 - 1	0.1 - 1
JC-2023-D	DWS1	SPD	11.3	0.23	0.23	0.1 - 1	0.1 - 1
JC-2023-D	DWS2	SPD	10.2	0.2	0.2	0.1 - 1	0.1 - 1
JC-2023-D	DWS3	SPD	5.6	0.11	0.11	0.1 - 1	0.1 - 1
ZR-2023-A	DWW	SPD	24	0.48	0.48	0.1 - 1	0.1 - 1
ZR-2023-A	DWS1	SPD	8.6	0.17	0.17	0.1 - 1	0.1 - 1
ZR-2023-A	DWS2	SPD	13.6	0.27	0.27	0.1 - 1	0.1 - 1
ZR-2023-A	DWS3	SPD	9.7	0.19	0.19	0.1 - 1	0.1 - 1
ZR-2023-B	DWW	SPD	1	0.02	0.02	0.01 - 0.1	0.01 - 0.1
ZR-2023-B	DWS1	SPD	21.7	0.43	0.43	0.1 - 1	0.1 - 1
ZR-2023-B	DWS2	SPD	13.7	0.27	0.27	0.1 - 1	0.1 - 1
ZR-2023-B	DWS3	SPD	13.4	0.27	0.27	0.1 - 1	0.1 - 1
ZR-2023-C	DWW	SPD	22.8	0.46	0.46	0.1 - 1	0.1 - 1
ZR-2023-C	DWS1	SPD	30.7	0.61	0.61	0.1 - 1	0.1 - 1
ZR-2023-C	DWS2	SPD	15.9	0.32	0.32	0.1 - 1	0.1 - 1
ZR-2023-C	DWS3	SPD	16.4	0.33	0.33	0.1 - 1	0.1 - 1
PC-2024-A	DWS1	SPD	24.97	0.5	0.5	0.1 - 1	0.1 - 1
PC-2024-A	DWS2	SPD	31.5	0.63	0.63	0.1 - 1	0.1 - 1
PC-2024-A	DWS3	SPD	16.8	0.34	0.34	0.1 - 1	0.1 - 1
PC-2024-B	DWS1	SPD	8.66	0.17	0.17	0.1 - 1	0.1 - 1
PC-2024-B	DWS2	SPD	12.05	0.24	0.24	0.1 - 1	0.1 - 1
PC-2024-B	DWS3	SPD	9.85	0.2	0.2	0.1 - 1	0.1 - 1
PC-2024-C	DWS1	SPD	39.65	0.79	0.79	0.1 - 1	0.1 - 1
PC-2024-C	DWS2	SPD	24.11	0.48	0.48	0.1 - 1	0.1 - 1
PC-2024-C	DWS3	SPD	14.85	0.3	0.3	0.1 - 1	0.1 - 1
JC-2024-B	DWW	SPD	43.88	0.88	0.88	0.1 - 1	0.1 - 1
ZR-2024-A	DWW	SPD	8.86	0.18	0.18	0.1 - 1	0.1 - 1
ZR-2024-A	DWS1	SPD	6.52	0.13	0.13	0.1 - 1	0.1 - 1
ZR-2024-A	DWS2	SPD	13.53	0.27	0.27	0.1 - 1	0.1 - 1
ZR-2024-A	DWS3	SPD	30.38	0.61	0.61	0.1 - 1	0.1 - 1
ZR-2024-B	DWW	SPD	12.65	0.25	0.25	0.1 - 1	0.1 - 1
ZR-2024-B	DWS1	SPD	16.67	0.33	0.33	0.1 - 1	0.1 - 1
ZR-2024-B	DWS2	SPD	18.26	0.37	0.37	0.1 - 1	0.1 - 1
ZR-2024-B	DWS3	SPD	61.72	1.23	1.23	> 1	> 1
ZR-2024-C	DWW	SPD	52.86	1.06	1.06	> 1	> 1
ZR-2024-C	DWS1	SPD	45.41	0.91	0.91	0.1 - 1	0.1 - 1
ZR-2024-C	DWS2	SPD	31.17	0.62	0.62	0.1 - 1	0.1 - 1
ZR-2024-C	DWS3	SPD	26.02	0.52	0.52	0.1 - 1	0.1 - 1
PC-2023-B	DWS1	SDZ	18.3	0	0.37	0.1 - 1	< 0.01

Sample ID	Site	Antibiotic	Concentration (ng L^{-1})	RQ_{ENV}	RQ_{AMR}	RQ_{AMR} (class)	RQ_{ENV} (class)
PC-2023-B	DWS2	SDZ	12.7	0	0.25	0.1 - 1	< 0.01
PC-2023-C	DWS1	SDZ	89.9	0.01	1.8	> 1	< 0.01
PC-2023-C	DWS2	SDZ	54.3	0	1.09	> 1	< 0.01
PC-2023-C	DWS3	SDZ	8.7	0	0.17	0.1 - 1	< 0.01
PC-2023-D	DWS1	SDZ	17.1	0	0.34	0.1 - 1	< 0.01
PC-2023-D	DWS2	SDZ	13.7	0	0.27	0.1 - 1	< 0.01
PC-2023-D	DWS3	SDZ	16.6	0	0.33	0.1 - 1	< 0.01
JC-2023-A	DWW	SDZ	29.1	0	0.58	0.1 - 1	< 0.01
JC-2023-A	DWS1	SDZ	19	0	0.38	0.1 - 1	< 0.01
JC-2023-A	DWS2	SDZ	17.5	0	0.35	0.1 - 1	< 0.01
JC-2023-A	DWS3	SDZ	19.5	0	0.39	0.1 - 1	< 0.01
JC-2023-B	DWW	SDZ	53.6	0	1.07	> 1	< 0.01
JC-2023-B	DWS1	SDZ	1.6	0	0.03	0.01 - 0.1	< 0.01
JC-2023-B	DWS2	SDZ	1.7	0	0.03	0.01 - 0.1	< 0.01
JC-2023-B	DWS3	SDZ	2.2	0	0.04	0.01 - 0.1	< 0.01
JC-2023-C	DWW	SDZ	73.7	0.01	1.47	> 1	< 0.01
JC-2023-C	DWS1	SDZ	16.1	0	0.32	0.1 - 1	< 0.01
JC-2023-C	DWS2	SDZ	13.3	0	0.27	0.1 - 1	< 0.01
JC-2023-C	DWS3	SDZ	6.7	0	0.13	0.1 - 1	< 0.01
JC-2023-D	DWW	SDZ	30.2	0	0.6	0.1 - 1	< 0.01
JC-2023-D	DWS1	SDZ	14.4	0	0.29	0.1 - 1	< 0.01
JC-2023-D	DWS2	SDZ	11	0	0.22	0.1 - 1	< 0.01
JC-2023-D	DWS3	SDZ	9.2	0	0.18	0.1 - 1	< 0.01
ZR-2023-A	DWW	SDZ	19.8	0	0.4	0.1 - 1	< 0.01
ZR-2023-B	DWS1	SDZ	17.1	0	0.34	0.1 - 1	< 0.01
ZR-2023-B	DWS2	SDZ	15.6	0	0.31	0.1 - 1	< 0.01
ZR-2023-B	DWS3	SDZ	10.8	0	0.22	0.1 - 1	< 0.01
ZR-2023-C	DWW	SDZ	133	0.01	2.66	> 1	< 0.01
ZR-2023-C	DWS1	SDZ	49.2	0	0.98	0.1 - 1	< 0.01
ZR-2023-C	DWS2	SDZ	19	0	0.38	0.1 - 1	< 0.01
ZR-2023-C	DWS3	SDZ	24.7	0	0.49	0.1 - 1	< 0.01
PC-2023-A	DWS1	OFC	46.7	0	0.02	0.01 - 0.1	< 0.01
PC-2023-A	DWS2	OFC	41.9	0	0	< 0.01	< 0.01
PC-2023-A	DWS3	OFC	61.4	0.01	0	< 0.01	< 0.01
PC-2023-B	DWS1	OFC	98.2	0.01	0.02	0.01 - 0.1	< 0.01
PC-2023-B	DWS2	OFC	52.1	0.01	0.01	< 0.01	< 0.01
PC-2023-B	DWS3	OFC	30.8	0	0.01	< 0.01	< 0.01
PC-2023-C	DWS1	OFC	295.6	0.03	0.03	0.01 - 0.1	0.01 - 0.1
PC-2023-C	DWS2	OFC	341.2	0.03	0.03	0.01 - 0.1	0.01 - 0.1
PC-2023-C	DWS3	OFC	155.9	0.02	0.03	0.01 - 0.1	0.01 - 0.1
PC-2023-D	DWS1	OFC	143.7	0.01	0.06	0.01 - 0.1	< 0.01
PC-2023-D	DWS2	OFC	212.2	0.02	0.01	< 0.01	0.01 - 0.1
PC-2023-D	DWS3	OFC	132.4	0.01	0.02	0.01 - 0.1	< 0.01
JC-2023-A	DWW	OFC	3.2	0	0.01	< 0.01	< 0.01
JC-2023-A	DWS1	OFC	2.1	0	0	< 0.01	< 0.01
JC-2023-A	DWS2	OFC	0.7	0	0	< 0.01	< 0.01
JC-2023-A	DWS3	OFC	0.5	0	0	< 0.01	< 0.01
JC-2023-B	DWW	OFC	1.3	0	0	< 0.01	< 0.01
JC-2023-B	DWS3	OFC	0.1	0	0	< 0.01	< 0.01
JC-2023-C	DWW	OFC	0.2	0	0	< 0.01	< 0.01
JC-2023-C	DWS1	OFC	0.7	0	0	< 0.01	< 0.01
JC-2023-C	DWS2	OFC	0.6	0	0	< 0.01	< 0.01
JC-2023-C	DWS3	OFC	0.1	0	0	< 0.01	< 0.01
JC-2023-D	DWS1	OFC	1.2	0	0	< 0.01	< 0.01
JC-2023-D	DWS2	OFC	3.1	0	0	< 0.01	< 0.01
JC-2023-D	DWS3	OFC	1.5	0	0	< 0.01	< 0.01
ZR-2023-A	DWW	OFC	257.9	0.03	0	< 0.01	0.01 - 0.1
ZR-2023-A	DWS1	OFC	45.4	0	0.03	0.01 - 0.1	< 0.01
ZR-2023-A	DWS2	OFC	90.4	0.01	0	< 0.01	< 0.01
ZR-2023-A	DWS3	OFC	12.8	0	0.01	< 0.01	< 0.01
ZR-2023-B	DWW	OFC	7.7	0	0	< 0.01	< 0.01

Sample ID	Site	Antibiotic	Concentration (ng L^{-1})	RQ_{ENV}	RQ_{AMR}	RQ_{AMR} (class)	RQ_{ENV} (class)
ZR-2023-B	DWS1	OFC	14.3	0	0	< 0.01	< 0.01
ZR-2023-B	DWS2	OFC	14.6	0	0	< 0.01	< 0.01
ZR-2023-B	DWS3	OFC	13	0	0	< 0.01	< 0.01
ZR-2023-C	DWW	OFC	147.7	0.01	0	< 0.01	< 0.01
ZR-2023-C	DWS1	OFC	36.9	0	0.01	< 0.01	< 0.01
ZR-2023-C	DWS2	OFC	63.3	0.01	0	< 0.01	< 0.01
ZR-2023-C	DWS3	OFC	21.5	0	0.01	< 0.01	< 0.01
PC-2024-A	DWS1	OFC	19.5	0	0.01	< 0.01	< 0.01
PC-2024-A	DWS2	OFC	10.35	0	0	< 0.01	< 0.01
PC-2024-A	DWS3	OFC	9.86	0	0	< 0.01	< 0.01
PC-2024-B	DWS1	OFC	13.23	0	0.01	< 0.01	< 0.01
PC-2024-B	DWS2	OFC	7.21	0	0	< 0.01	< 0.01
PC-2024-B	DWS3	OFC	7.62	0	0	< 0.01	< 0.01
PC-2024-C	DWS1	OFC	9.56	0	0.01	< 0.01	< 0.01
PC-2024-C	DWS2	OFC	5.22	0	0	< 0.01	< 0.01
PC-2024-C	DWS3	OFC	3.94	0	0	< 0.01	< 0.01
JC-2024-A	DWW	OFC	3.14	0	0	< 0.01	< 0.01
JC-2024-A	DWS1	OFC	2.48	0	0	< 0.01	< 0.01
JC-2024-A	DWS2	OFC	2.83	0	0	< 0.01	< 0.01
JC-2024-A	DWS3	OFC	3.69	0	0	< 0.01	< 0.01
JC-2024-B	DWW	OFC	2.79	0	0	< 0.01	< 0.01
ZR-2024-A	DWW	OFC	175.3	0.02	0	< 0.01	0.01 - 0.1
ZR-2024-A	DWS1	OFC	3.93	0	0.02	0.01 - 0.1	< 0.01
ZR-2024-A	DWS2	OFC	12.12	0	0	< 0.01	< 0.01
ZR-2024-A	DWS3	OFC	12.24	0	0	< 0.01	< 0.01
ZR-2024-B	DWW	OFC	823.19	0.08	0	< 0.01	0.01 - 0.1
ZR-2024-B	DWS1	OFC	35.19	0	0.08	0.01 - 0.1	< 0.01
ZR-2024-B	DWS2	OFC	26.01	0	0	< 0.01	< 0.01
ZR-2024-B	DWS3	OFC	28.19	0	0	< 0.01	< 0.01
ZR-2024-C	DWW	OFC	300.52	0.03	0	< 0.01	0.01 - 0.1
ZR-2024-C	DWS1	OFC	53.56	0.01	0.03	0.01 - 0.1	< 0.01
ZR-2024-C	DWS2	OFC	10.2	0	0.01	< 0.01	< 0.01
ZR-2024-C	DWS3	OFC	8.27	0	0	< 0.01	< 0.01
PC-2023-A	DWS1	CFC	69.6	0.15	1.16	> 1	0.1 - 1
PC-2023-A	DWS2	CFC	48.6	0.11	0.81	0.1 - 1	0.1 - 1
PC-2023-A	DWS3	CFC	131.5	0.29	2.19	> 1	0.1 - 1
PC-2023-B	DWS1	CFC	58.4	0.13	0.97	0.1 - 1	0.1 - 1
PC-2023-B	DWS2	CFC	52	0.12	0.87	0.1 - 1	0.1 - 1
PC-2023-B	DWS3	CFC	45.2	0.1	0.75	0.1 - 1	0.01 - 0.1
PC-2023-C	DWS1	CFC	216.8	0.48	3.61	> 1	0.1 - 1
PC-2023-C	DWS2	CFC	156	0.35	2.6	> 1	0.1 - 1
PC-2023-C	DWS3	CFC	62.9	0.14	1.05	> 1	0.1 - 1
PC-2023-D	DWS1	CFC	331.2	0.74	5.52	> 1	0.1 - 1
PC-2023-D	DWS2	CFC	112.3	0.25	1.87	> 1	0.1 - 1
PC-2023-D	DWS3	CFC	68	0.15	1.13	> 1	0.1 - 1
JC-2023-A	DWW	CFC	33.1	0.07	0.55	0.1 - 1	0.01 - 0.1
JC-2023-A	DWS1	CFC	28.7	0.06	0.48	0.1 - 1	0.01 - 0.1
JC-2023-A	DWS2	CFC	22.2	0.05	0.37	0.1 - 1	0.01 - 0.1
JC-2023-A	DWS3	CFC	13.2	0.03	0.22	0.1 - 1	0.01 - 0.1
JC-2023-B	DWW	CFC	10.6	0.02	0.18	0.1 - 1	0.01 - 0.1
JC-2023-C	DWW	CFC	6.6	0.01	0.11	0.1 - 1	< 0.01
JC-2023-C	DWS1	CFC	4.7	0.01	0.08	0.01 - 0.1	< 0.01
JC-2023-C	DWS2	CFC	4.4	0.01	0.07	0.01 - 0.1	< 0.01
JC-2023-C	DWS3	CFC	2.2	0	0.04	0.01 - 0.1	< 0.01
JC-2023-D	DWW	CFC	11.3	0.03	0.19	0.1 - 1	0.01 - 0.1
JC-2023-D	DWS1	CFC	9.2	0.02	0.15	0.1 - 1	0.01 - 0.1
JC-2023-D	DWS2	CFC	9.1	0.02	0.15	0.1 - 1	0.01 - 0.1
JC-2023-D	DWS3	CFC	2.2	0	0.04	0.01 - 0.1	< 0.01
ZR-2023-A	DWW	CFC	22.6	0.05	0.38	0.1 - 1	0.01 - 0.1
ZR-2023-A	DWS1	CFC	6.8	0.02	0.11	0.1 - 1	0.01 - 0.1
ZR-2023-A	DWS2	CFC	7.4	0.02	0.12	0.1 - 1	0.01 - 0.1

Sample ID	Site	Antibiotic	Concentration (ng L^{-1})	RQ_{ENV}	RQ_{AMR}	RQ_{AMR} (class)	RQ_{ENV} (class)
ZR-2023-A	DWS3	CFC	2.6	0.01	0.04	0.01 - 0.1	< 0.01
ZR-2023-B	DWW	CFC	0	0	0	< 0.01	< 0.01
ZR-2023-B	DWS1	CFC	5.6	0.01	0.09	0.01 - 0.1	< 0.01
ZR-2023-B	DWS2	CFC	3.1	0.01	0.05	0.01 - 0.1	< 0.01
ZR-2023-C	DWW	CFC	221.8	0.49	3.7	> 1	0.1 - 1
ZR-2023-C	DWS1	CFC	77.9	0.17	1.3	> 1	0.1 - 1
ZR-2023-C	DWS2	CFC	43	0.1	0.72	0.1 - 1	0.01 - 0.1
ZR-2023-C	DWS3	CFC	22.9	0.05	0.38	0.1 - 1	0.01 - 0.1
PC-2024-A	DWS1	CFC	2.39	0.01	0.04	0.01 - 0.1	< 0.01
PC-2024-A	DWS2	CFC	1.71	0	0.03	0.01 - 0.1	< 0.01
PC-2024-A	DWS3	CFC	0.99	0	0.02	0.01 - 0.1	< 0.01
PC-2024-C	DWS1	CFC	18.23	0.04	0.3	0.1 - 1	0.01 - 0.1
PC-2024-C	DWS2	CFC	4.47	0.01	0.07	0.01 - 0.1	< 0.01
PC-2024-C	DWS3	CFC	2.26	0.01	0.04	0.01 - 0.1	< 0.01
ZR-2024-A	DWW	CFC	29.53	0.07	0.49	0.1 - 1	0.01 - 0.1
ZR-2024-B	DWW	CFC	35.32	0.08	0.59	0.1 - 1	0.01 - 0.1
ZR-2024-B	DWS1	CFC	12.26	0.03	0.2	0.1 - 1	0.01 - 0.1
ZR-2024-B	DWS2	CFC	4.74	0.01	0.08	0.01 - 0.1	< 0.01
ZR-2024-B	DWS3	CFC	2.77	0.01	0.05	0.01 - 0.1	< 0.01
ZR-2024-C	DWW	CFC	129.09	0.29	2.15	> 1	0.1 - 1
ZR-2024-C	DWS1	CFC	10.78	0.02	0.18	0.1 - 1	0.01 - 0.1
ZR-2024-C	DWS2	CFC	4.27	0.01	0.07	0.01 - 0.1	< 0.01
ZR-2024-C	DWS3	CFC	1.95	0	0.03	0.01 - 0.1	< 0.01
JC-2023-A	DWW	NFC	62.2	0.000518	0.12	0.1 - 1	< 0.01
JC-2023-A	DWS1	NFC	63.5	0.000529	0.13	0.1 - 1	< 0.01
JC-2023-A	DWS2	NFC	67.7	0.000564	0.14	0.1 - 1	< 0.01
JC-2023-A	DWS3	NFC	63.8	0.000532	0.13	0.1 - 1	< 0.01
ZR-2023-A	DWW	NFC	66.8	0.000557	0.13	0.1 - 1	< 0.01
ZR-2023-A	DWS1	NFC	57.9	0.000483	0.12	0.1 - 1	< 0.01
ZR-2023-A	DWS2	NFC	71.9	0.000599	0.14	0.1 - 1	< 0.01
ZR-2023-A	DWS3	NFC	43.6	0.000363	0.09	0.01 - 0.1	< 0.01
ZR-2023-B	DWW	NFC	43.7	0.000364	0.09	0.01 - 0.1	< 0.01
ZR-2023-B	DWS1	NFC	64.2	0.000535	0.13	0.1 - 1	< 0.01
ZR-2023-B	DWS2	NFC	71	0.000592	0.14	0.1 - 1	< 0.01
ZR-2023-B	DWS3	NFC	62.3	0.000519	0.12	0.1 - 1	< 0.01
ZR-2023-C	DWW	NFC	48.8	0.000407	0.1	0.01 - 0.1	< 0.01
ZR-2023-C	DWS1	NFC	101.7	0.000848	0.2	0.1 - 1	< 0.01
ZR-2023-C	DWS2	NFC	104.2	0.000868	0.21	0.1 - 1	< 0.01
ZR-2023-C	DWS3	NFC	37	0.000308	0.07	0.01 - 0.1	< 0.01
PC-2023-A	DWS1	TMP	19.9	0	0.04	0.01 - 0.1	< 0.01
PC-2023-A	DWS2	TMP	22.8	0	0.05	0.01 - 0.1	< 0.01
PC-2023-A	DWS3	TMP	33.5	0	0.07	0.01 - 0.1	< 0.01
PC-2023-B	DWS1	TMP	131.8	0	0.26	0.1 - 1	< 0.01
PC-2023-B	DWS2	TMP	32.9	0	0.07	0.01 - 0.1	< 0.01
PC-2023-B	DWS3	TMP	40.5	0	0.08	0.01 - 0.1	< 0.01
PC-2023-C	DWS1	TMP	66.5	0	0.13	0.1 - 1	< 0.01
PC-2023-C	DWS2	TMP	32	0	0.06	0.01 - 0.1	< 0.01
PC-2023-C	DWS3	TMP	25.3	0	0.05	0.01 - 0.1	< 0.01
PC-2023-D	DWS1	TMP	27.1	0	0.05	0.01 - 0.1	< 0.01
PC-2023-D	DWS2	TMP	11.8	0	0.02	0.01 - 0.1	< 0.01
PC-2023-D	DWS3	TMP	11.2	0	0.02	0.01 - 0.1	< 0.01
JC-2023-A	DWS1	TMP	2.2	0	0	< 0.01	< 0.01
JC-2023-A	DWS2	TMP	1.6	0	0	< 0.01	< 0.01
JC-2023-A	DWS3	TMP	3.3	0	0.01	< 0.01	< 0.01
JC-2023-C	DWW	TMP	1.1	0	0	< 0.01	< 0.01
JC-2023-C	DWS1	TMP	1.3	0	0	< 0.01	< 0.01
JC-2023-C	DWS2	TMP	0.5	0	0	< 0.01	< 0.01
JC-2023-C	DWS3	TMP	1.9	0	0	< 0.01	< 0.01
ZR-2023-A	DWW	TMP	107	0	0.21	0.1 - 1	< 0.01
ZR-2023-A	DWS1	TMP	31.5	0	0.06	0.01 - 0.1	< 0.01
ZR-2023-A	DWS2	TMP	55.5	0	0.11	0.1 - 1	< 0.01

Sample ID	Site	Antibiotic	Concentration (ng L^{-1})	RQ_{ENV}	RQ_{AMR}	RQ_{AMR} (class)	RQ_{ENV} (class)
ZR-2023-A	DWS3	TMP	5.3	0	0.01	< 0.01	< 0.01
ZR-2023-B	DWW	TMP	8.4	0	0.02	0.01 - 0.1	< 0.01
ZR-2023-B	DWS1	TMP	75.8	0	0.15	0.1 - 1	< 0.01
ZR-2023-B	DWS2	TMP	70.2	0	0.14	0.1 - 1	< 0.01
ZR-2023-B	DWS3	TMP	41.9	0	0.08	0.01 - 0.1	< 0.01
ZR-2023-C	DWW	TMP	444.1	0	0.89	0.1 - 1	< 0.01
ZR-2023-C	DWS1	TMP	193.5	0	0.39	0.1 - 1	< 0.01
ZR-2023-C	DWS2	TMP	124.8	0	0.25	0.1 - 1	< 0.01
ZR-2023-C	DWS3	TMP	152.6	0	0.31	0.1 - 1	< 0.01
PC-2024-A	DWS1	TMP	13.38	0	0.03	0.01 - 0.1	< 0.01
PC-2024-A	DWS2	TMP	14.69	0	0.03	0.01 - 0.1	< 0.01
PC-2024-A	DWS3	TMP	11.4	0	0.02	0.01 - 0.1	< 0.01
PC-2024-B	DWS1	TMP	9.28	0	0.02	0.01 - 0.1	< 0.01
PC-2024-B	DWS2	TMP	10.12	0	0.02	0.01 - 0.1	< 0.01
PC-2024-B	DWS3	TMP	12.7	0	0.03	0.01 - 0.1	< 0.01
PC-2024-C	DWS1	TMP	23.69	0	0.05	0.01 - 0.1	< 0.01
PC-2024-C	DWS2	TMP	27.09	0	0.05	0.01 - 0.1	< 0.01
PC-2024-C	DWS3	TMP	6.82	0	0.01	< 0.01	< 0.01
JC-2024-A	DWW	TMP	4.99	0	0.01	< 0.01	< 0.01
JC-2024-A	DWS1	TMP	4.9	0	0.01	< 0.01	< 0.01
JC-2024-A	DWS2	TMP	4.69	0	0.01	< 0.01	< 0.01
JC-2024-A	DWS3	TMP	4.51	0	0.01	< 0.01	< 0.01
JC-2024-B	DWW	TMP	2.19	0	0	< 0.01	< 0.01
JC-2024-B	DWS1	TMP	5.4	0	0.01	< 0.01	< 0.01
JC-2024-B	DWS2	TMP	6.21	0	0.01	< 0.01	< 0.01
JC-2024-B	DWS3	TMP	14.65	0	0.03	0.01 - 0.1	< 0.01
ZR-2024-A	DWW	TMP	520.5	0	1.04	> 1	< 0.01
ZR-2024-A	DWS1	TMP	10.11	0	0.02	0.01 - 0.1	< 0.01
ZR-2024-A	DWS2	TMP	22.6	0	0.05	0.01 - 0.1	< 0.01
ZR-2024-A	DWS3	TMP	20.76	0	0.04	0.01 - 0.1	< 0.01
ZR-2024-B	DWW	TMP	402.03	0	0.8	0.1 - 1	< 0.01
ZR-2024-B	DWS1	TMP	45.23	0	0.09	0.01 - 0.1	< 0.01
ZR-2024-B	DWS2	TMP	37.41	0	0.07	0.01 - 0.1	< 0.01
ZR-2024-B	DWS3	TMP	30.23	0	0.06	0.01 - 0.1	< 0.01
ZR-2024-C	DWW	TMP	170.53	0	0.34	0.1 - 1	< 0.01
ZR-2024-C	DWS1	TMP	59.39	0	0.12	0.1 - 1	< 0.01
ZR-2024-C	DWS2	TMP	21.82	0	0.04	0.01 - 0.1	< 0.01
ZR-2024-C	DWS3	TMP	10.23	0	0.02	0.01 - 0.1	< 0.01
PC-2023-B	DWS1	AZMC	2.8	0.09	0.01	< 0.01	0.01 - 0.1
PC-2023-B	DWS2	AZMC	1	0.03	0	< 0.01	0.01 - 0.1
PC-2023-B	DWS3	AZMC	0.7	0.02	0	< 0.01	0.01 - 0.1
ZR-2023-A	DWW	AZMC	2.3	0.08	0.01	< 0.01	0.01 - 0.1
ZR-2023-A	DWS2	AZMC	0.9	0.03	0	< 0.01	0.01 - 0.1
ZR-2023-A	DWS3	AZMC	0.7	0.02	0	< 0.01	0.01 - 0.1
ZR-2023-B	DWS1	AZMC	2.3	0.08	0.01	< 0.01	0.01 - 0.1
ZR-2023-B	DWS2	AZMC	2.1	0.07	0.01	< 0.01	0.01 - 0.1
ZR-2023-B	DWS3	AZMC	1.5	0.05	0.01	< 0.01	0.01 - 0.1
ZR-2024-A	DWW	AZMC	344.46	11.48	1.38	> 1	> 1
ZR-2024-A	DWS1	AZMC	3.91	0.13	0.02	0.01 - 0.1	0.1 - 1
ZR-2024-A	DWS2	AZMC	7.46	0.25	0.03	0.01 - 0.1	0.1 - 1
ZR-2024-A	DWS3	AZMC	4.63	0.15	0.02	0.01 - 0.1	0.1 - 1
ZR-2024-B	DWW	AZMC	232.53	7.75	0.93	0.1 - 1	> 1
ZR-2024-B	DWS1	AZMC	7.3	0.24	0.03	0.01 - 0.1	0.1 - 1
ZR-2024-B	DWS2	AZMC	6.63	0.22	0.03	0.01 - 0.1	0.1 - 1
ZR-2024-B	DWS3	AZMC	7.31	0.24	0.03	0.01 - 0.1	0.1 - 1
ZR-2024-C	DWW	AZMC	216.76	7.23	0.87	0.1 - 1	> 1
ZR-2024-C	DWS1	AZMC	97.76	3.26	0.39	0.1 - 1	> 1
ZR-2024-C	DWS2	AZMC	16.58	0.55	0.07	0.01 - 0.1	0.1 - 1
ZR-2024-C	DWS3	AZMC	10.7	0.36	0.04	0.01 - 0.1	0.1 - 1
ZR-2023-A	DWW	CRMC	2	0.008	0.008	< 0.01	< 0.01
ZR-2023-A	DWS2	CRMC	0.86	0.00344	0.00344	< 0.01	< 0.01

Sample ID	Site	Antibiotic	Concentration (ng L^{-1})	RQ_{ENV}	RQ_{AMR}	RQ_{AMR} (class)	RQ_{ENV} (class)
ZR-2023-A	DWS3	CRMC	0.68	0.00272	0.00272	< 0.01	< 0.01
ZR-2023-B	DWS1	CRMC	2.3	0.0092	0.0092	< 0.01	< 0.01
ZR-2023-B	DWS2	CRMC	2.3	0.0092	0.0092	< 0.01	< 0.01
ZR-2023-B	DWS3	CRMC	1.7	0.0068	0.0068	< 0.01	< 0.01
PC-2024-C	DWS1	CRMC	2.8	0.01	0.01	< 0.01	< 0.01
PC-2024-C	DWS2	CRMC	2.32	0.01	0.01	< 0.01	< 0.01
PC-2024-C	DWS3	CRMC	3.5	0.01	0.01	< 0.01	< 0.01
ZR-2024-A	DWW	CRMC	27.98	0.11	0.11	0.1 - 1	0.1 - 1
ZR-2024-A	DWS1	CRMC	0.5	0	0	< 0.01	< 0.01
ZR-2024-A	DWS2	CRMC	1.27	0.01	0.01	< 0.01	< 0.01
ZR-2024-A	DWS3	CRMC	1.27	0.01	0.01	< 0.01	< 0.01
ZR-2024-B	DWW	CRMC	35.17	0.14	0.14	0.1 - 1	0.1 - 1
ZR-2024-B	DWS1	CRMC	6.19	0.02	0.02	0.01 - 0.1	0.01 - 0.1
ZR-2024-B	DWS2	CRMC	2.24	0.01	0.01	< 0.01	< 0.01
ZR-2024-B	DWS3	CRMC	4.76	0.02	0.02	0.01 - 0.1	0.01 - 0.1
ZR-2024-C	DWW	CRMC	56.42	0.23	0.23	0.1 - 1	0.1 - 1
ZR-2024-C	DWS1	CRMC	20.3	0.08	0.08	0.01 - 0.1	0.01 - 0.1
ZR-2024-C	DWS2	CRMC	2.77	0.01	0.01	< 0.01	< 0.01
ZR-2024-C	DWS3	CRMC	3.93	0.02	0.02	0.01 - 0.1	0.01 - 0.1
PC-2024-A	DWS1	AMX	72.22	0.29	0.29	0.1 - 1	0.1 - 1
PC-2024-A	DWS2	AMX	35.27	0.14	0.14	0.1 - 1	0.1 - 1
PC-2024-A	DWS3	AMX	14.7	0.06	0.06	0.01 - 0.1	0.01 - 0.1

The RQ calculated values were categorized into four levels: no risk (RQ < 0.01), low risk (0.01 < RQ < 0.1), moderate risk (0.1 < RQ < 1), and high risk (RQ > 1).

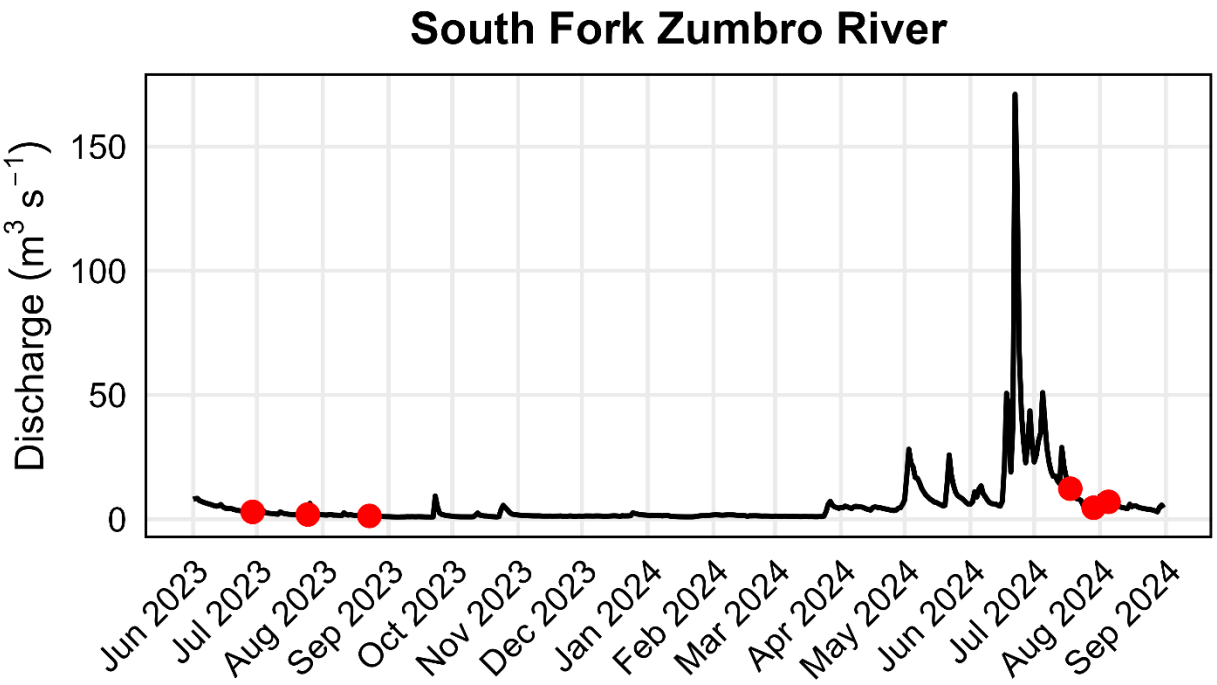


Figure S1. Hydrograph for the South Fork Zumbro River at USGS 5372995 [11] between 2023 and 2024. Sample collection dates are plotted as red filled circular symbols for the South Fork Zumbro River.

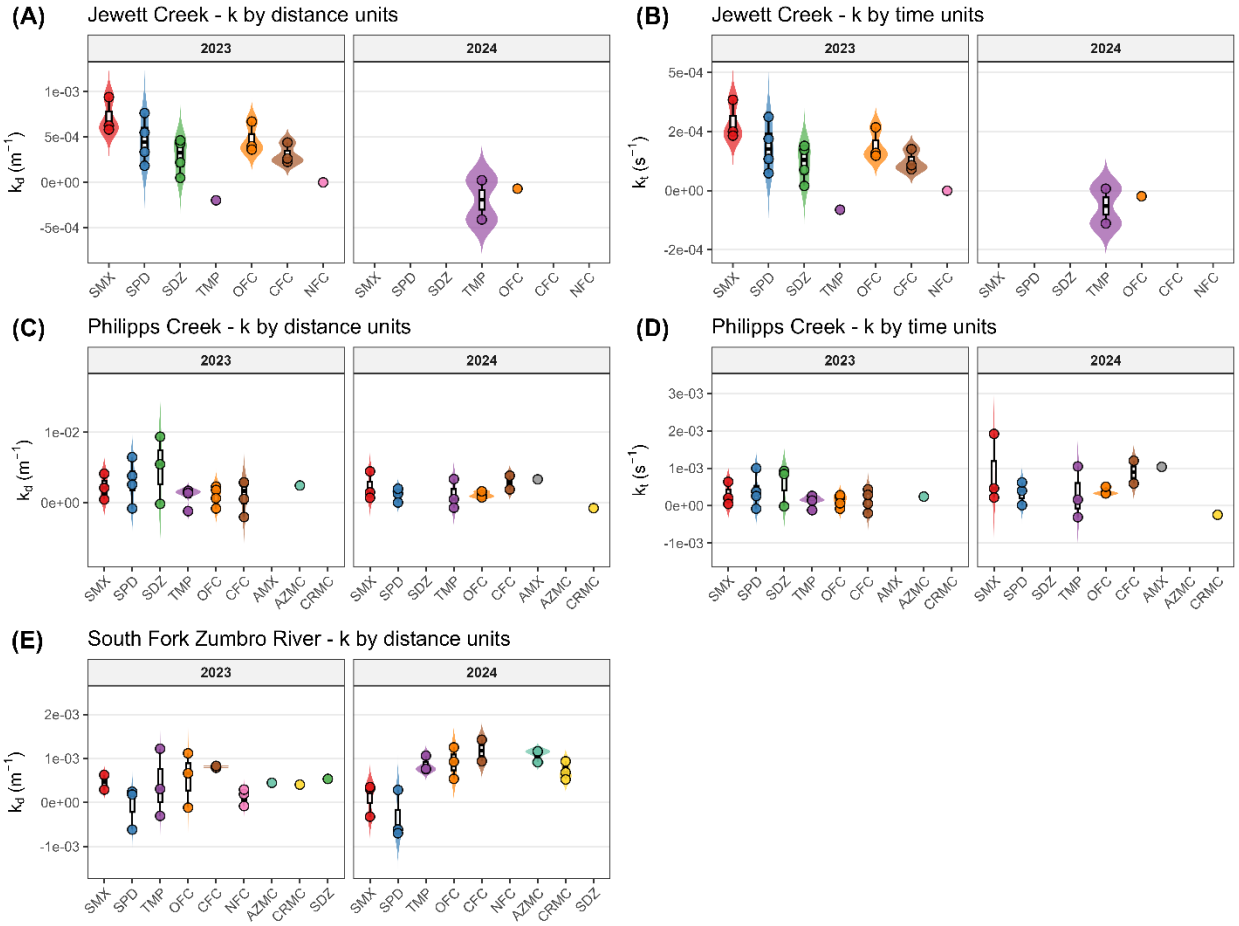


Figure S2. Plot distribution of in-stream attenuation rate constants, k_d and k_t, for antibiotics measured in Jewett Creek (A and B, respectively), Phillips Creek (C and D, respectively), and the South Fork Zumbro River (C) for 2023 and 2024. Each plot represents the full distribution of *k* with observation >2, boxplots indicate the median and 10th and 90th interquartile ranges, and individual points show each level of *k*. Single observations are also shown as circles. Higher *k* indicates faster attenuation with distance downstream, whereas negative values suggest increasing concentration with distance.

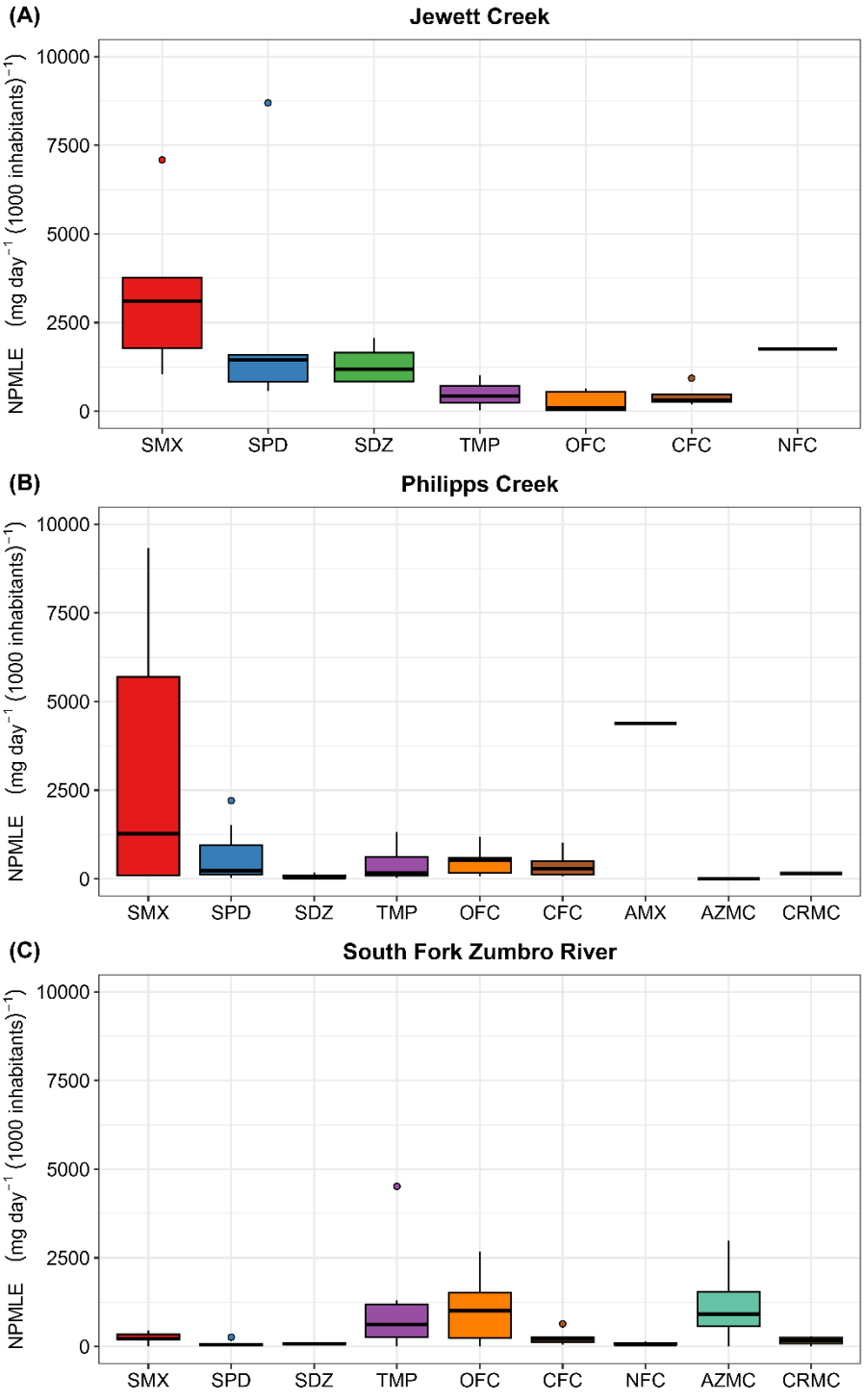


Figure S3. Box plot of NPMLE (mg per day per 1,000 inhabitants) for nine antibiotics detected in the DWW and DWS1 across Jewett Creek, Phillips Creek, and South Fork Zumbro River between 2023 and 2024. The middle line in each box represents the median and the points outliers. The South Fork of the Zumbro River sewershed encompasses a larger area/population and also a large hospital.

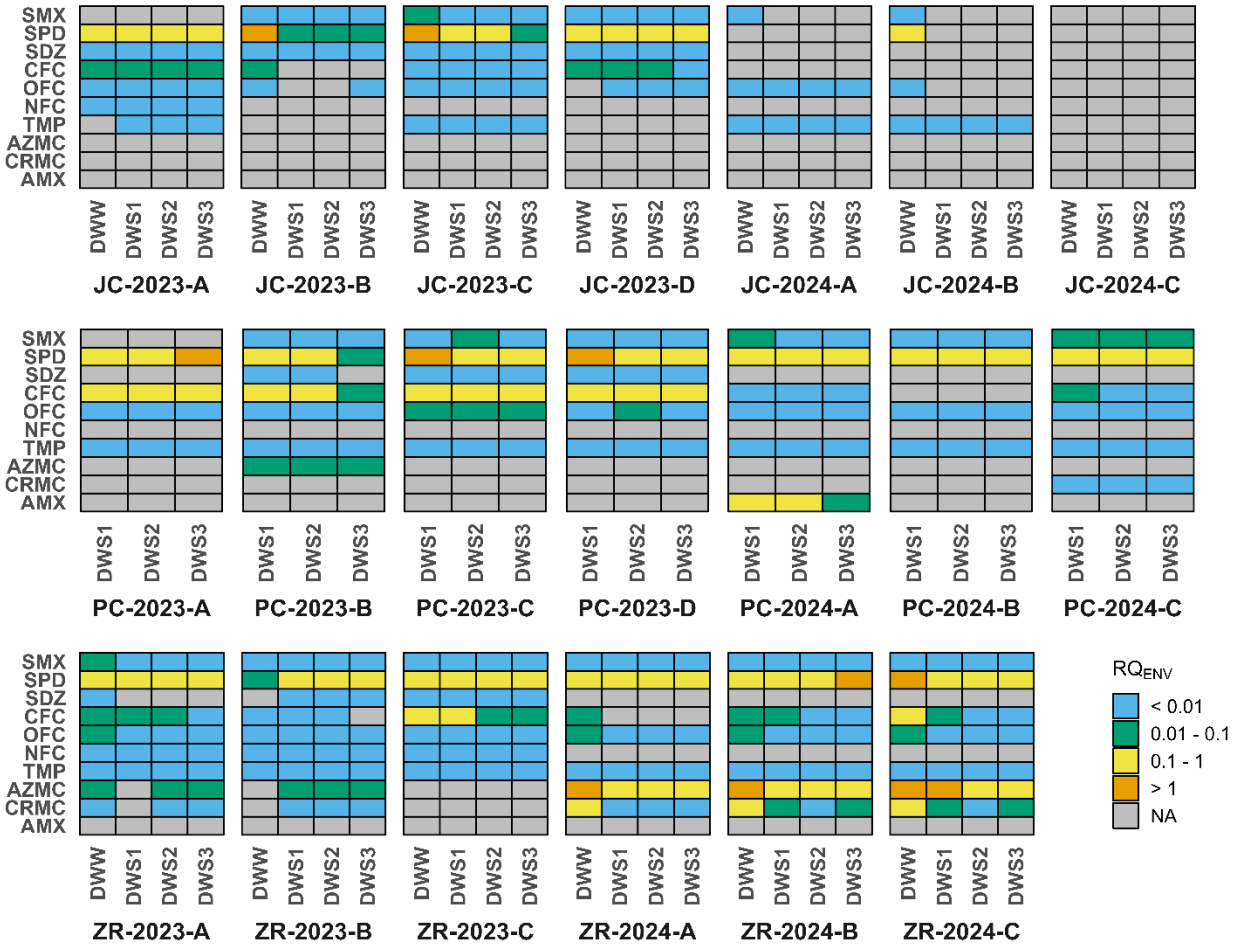


Figure S4. Heatmap of RQ_{ENV} of ten antibiotics calculated based on eco-toxicology for species in sampling sites in Jewett Creek, Philipps Creek, and South Fork Zumbro River during spring and summer of 2023 and 2024. The RQ calculated values were categorized into four levels: no risk (RQ < 0.01), low risk (0.01 < RQ < 0.1), moderate risk (0.1 < RQ < 1), and high risk (RQ > 1). NA: antibiotics were not detected in that sampling event.

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