

Supporting Information

Text S1 Pretreatment of water samples for antibiotic detection

Water samples were filtered through 0.7 μm muffle furnace-burned glass fiber filter to remove suspended solids, then 500 mg Na_2EDTA was added to the filtrate to complex divalent cations and the pH value was then adjusted to 3 with HCL (6 mol/L). Oasis hydrophilic–lipophilic balance (HLB) cartridges (6 mL/500 mg, Waters, UK) were used to gather antibiotics using the Supelco Visiprep SPE system (Supelco, USA) with a loading rate of 1–2 mL/min, then pretreated with 5 mL methanol followed by 5 mL ultrapure water three times. The HLB cartridges were then washed with 10 mL ultrapure water and lyophilized (Labconco Freeze Dry System, USA) for at least 8 h. Finally eluted with 3×2 mL of methanol three times. The eluate was collected into a glass tube and evaporated to dryness using a pressure gas blowing concentrator (Anpel, China). Next, 1 mL methanol containing 100 ng of each internal standard substance was added to redissolve the target antibiotics (Englert, 2007).

Text S2 qPCR procedure for *sul1*, *sul2*, *intI1*, 16S-rDNA modified from Wu et al. (2010)

The total volume of reaction system is 20 μL , containing 10 μL PCR mix (TIANGEN, China), 7 μL ddH₂O, 1 μL of each 10 $\mu\text{mol/L}$ primer, and 1 μL DNA template. The reaction procedure is summarized as initial denaturation at 95°C for 3 min; 40 cycles of 30 s at 95°C, 30 s of annealing at a specified temperature for each gene, and extension for 1 min at 72°C; with a final extension at 72°C for 5 min.

Table S1 Surface water samples information

Number	Name	Latitude and Longitude	Distance from recharge	Sample Type	Remarks
1	SZ	116°37'57.89" 40°08'43.57"	Recharge point	Surface water	Tributary
2	JH	116°38'01.60" 40°08'40.62"	0.15 km	Surface water	
3	CQ	116°38'16.82" 40°08'34.60"	0.6 km	Surface water	
4	JC	116°38'46.71" 40°08'33.18"	1.5 km	Surface water	
5	SX	116°39'21.24" 40°08'39.97"	2.5 km	Surface water	
6	ZY	116°39'47.74" 40°08'22.49"	3.0 km	Surface water	
7	ZK	116°39'57.93" 40°08'14.64"	3.5 km	Surface water	
8	GY	116°40'02.22" 40°08'10.03"	3.8 km	Surface water	
9	JCK	116°40'11.76" 40°07'58.87"	4.5 km	Surface water	Intersection of the two rivers
10	FB	116°40'19.64" 40°07'42.39"	5.2 km	Surface water	
11	DQ	116°41'22.44" 40°07'06.06"	7.0 km	Surface water	
12	XJB	116°41'53.88" 40°06'56.20"	8.0 km	Surface water	
13	TB	116°40'23.77" 40°08'13.10"	4.2 km	Surface water	An artificial DAMS
14	XYZ	116°40'44.57" 40°10'13.29"	8.0 km	Surface water	Sluice shut down for years

Table S2 Standard curves of target antibiotics

Antibiotics	Standard curves	R^2	Linearity range
SMX	$y = 162.84x + 168.76$	0.9991	1–50
TMP	$y = 646.78x + 302.42$	0.9989	1–50
SDZ	$y = 150.84x + 266.52$	0.9983	1–50
SML	$y = 257.09x + 176.18$	0.9993	1–50
SDM	$y = 341.18x + 540.15$	0.9987	1–50
STZ	$y = 210.23x + 281.64$	0.9969	1–50

Table S3 Recovery, LOD and LOQ of target antibiotics

Chemicals	Recovery (%)	LOD (ng/L)	LOQ (ng/L)
SMX	89.2 ± 3.04	1.36	0.45
TMP	116.0 ± 6.72	0.69	0.23
SDZ	101.8 ± 3.68	1.31	0.43
SML	88.8 ± 6.22	0.99	0.33
SDM	115.9 ± 6.01	0.58	0.19
STZ	87.1 ± 7.50	1.43	0.47

Table S4 Primers and annealing temperatures for qPCR

Types	Genes	Primer	Sequence (5'-3')	PCR annealing temp (°C)	Amplicon size (bp)	Reference
Bacteria	16S-rDNA	1369F	CGGTGAATACGTTCYCGG	52	123	Suzuki et al. (2000)
		1492R	GGWTACCTTGTTACGACTT			
Mobile genetic elements	<i>intI1</i>	FW	GGCTTCGTGATGCCTGCTT	58	146	Luo et al. (2010)
		RW	CATTCCTGGCCGTGGTTCT			
Sulfonamide resistance genes	<i>sul1</i>	FW	CGCACCGGAAACATCGCTGCAC	65	163	Pei et al. (2006)
		RW	TGAAGTTCCGCCCAAGGCTCG			
	<i>sul2</i>	FW	TCCGGTGGAGGCCGGTATCTGG	64	191	Pei et al. (2006)
		RW	CGGGAATGCCATCTGCCTTGAG			

Table S5 Standard Application Curves of ARGs and 16S-rDNA

Target	Standard curve	Efficiency (%)	R^2	T_m (°C)
<i>sul1</i>	$y = -3.204x + 37.569$	105.154	0.998	88.24
16S-r DNA	$y = -3.2184x + 34.084$	97.102	0.997	86.01
<i>sul2</i>	$y = -3.42x + 35.767$	96.079	0.998	91.70
16S-r RNA	$y = -3.3172x + 36.4226$	98.214	0.997	86.01
<i>intI1</i>	$y = -3.41x + 35.219$	96.467	0.998	88.38
16S-r RNA	$y = -3.329x + 39.203$	99.711	0.998	86.01

Table S6 Water quality about the samples

WQ	Time	SZ	JH	CQ	JC	SX	ZY	ZK	GY	JCK	FB	DQ	XJB	TB	XYZ
pH	12.15.2016	7.70	7.47	–	–	7.77	–	–	–	7.78	8.04	–	7.54	–	–
	03.07.2017	7.83	7.67	7.61	7.91	8.19	8.39	8.33	8.42	8.47	8.42	7.83	8.27	8.44	8.11
	06.09.2017	7.99	7.91	8.01	7.95	8.09	8.62	8.78	8.89	8.94	9.47	9.92	9.76	8.96	9.03
	09.19.2017	7.70	7.67	7.56	7.53	7.59	8.58	8.80	9.01	9.02	8.60	9.97	9.78	8.65	8.05
EC ($\mu\text{s}/\text{cm}$)	12.15.2016	1130	889	–	–	862	–	–	–	817	862	–	728	–	–
	03.07.2017	1093	1040	939	946	910	914	873	868	829	919	887	871	888	150
	06.09.2017	1110	959	953	952	958	937	923	888	866	805	762	749	869	870
TOC (mg/L)	09.19.2017	780	792	802	779	781	799	781	743	730	688	464	440	697	576
	12.15.2016	80.047	19.855	–	–	22.735	–	26.590	–	21.820	–	17.290	–	–	–
	03.07.2017	76.394	13.442	11.810	22.171	9.484	7.191	6.818	7.135	9.085	13.209	8.697	7.717	7.669	69.098
	06.09.2017	16.891	5.412	7.288	5.365	5.199	50.64	47.470	41.460	42.220	17.590	9.845	12.020	9.754	8.379
COD (mg/L)	09.19.2017	101.700	57.090	52.510	61.450	59.500	51.000	49.860	51.970	44.000	44.180	25.520	27.250	49.200	45.480
	12.15.2016	35	18	–	–	7.0	–	–	–	17	18	–	19	–	–
	03.07.2017	43	12	19	56	24	22	17	19	29	39	28	24	23	9.5
	06.09.2017	18	8	17	10	18	17	15	11	17	54	90	25	14	16
TP (mg/L)	09.19.2017	18	9	20	8	18	14	12	23	20	17	59	42	14	29
	12.15.2016	2.500	0.910	–	–	0.570	–	–	–	0.860	1.720	–	0.460	–	–
	03.07.2017	1.950	0.490	0.490	0.470	0.530	0.640	0.280	0.360	0.440	0.340	1.120	0.440	0.650	0.460
	06.09.2017	1.440	0.500	1.130	ND	0.430	0.250	0.260	0.410	0.380	0.980	1.010	0.740	0.260	0.360
NO ₂ ⁻ (mg/L)	09.19.2017	2.130	0.740	0.650	0.730	1.190	0.970	1.020	0.800	0.690	0.620	0.690	0.750	0.670	1.000
	12.15.2016	0.004	0.010	–	–	0.019	–	–	–	0.024	0.035	–	0.095	–	–
	03.07.2017	0.017	0.004	0.031	0.016	0.019	0.032	0.070	0.030	0.028	0.037	0.075	0.037	0.028	0.225
	06.09.2017	0.013	0.037	0.053	0.061	0.096	0.094	0.089	0.084	0.079	0.235	0.021	0.016	0.083	0.053

	09.19.2017	0.020	0.002	0.008	0.023	0.048	0.057	0.053	0.024	0.044	0.060	ND	ND	0.068	0.005
NH ₄ ⁺	12.15.2016	14.970	0.098	–	–	0.169	–	–	–	0.289	0.190	–	0.418	–	–
(mg/L)	03.07.2017	13.755	0.280	0.130	0.055	0.105	0.130	0.685	0.175	0.155	0.100	4.720	0.430	0.145	0.020
	06.09.2017	10.269	0.362	0.680	0.397	0.276	0.402	0.480	0.415	0.601	1.962	1.275	0.950	0.603	0.618
	09.19.2017	13.815	0.044	0.082	0.015	0.158	0.175	0.190	0.370	0.260	0.133	0.057	0.030	0.115	0.340
NO ₃ ⁻	12.15.2016	ND	13.925	–	–	13.545	–	–	–	2.492	12.340	–	2.151	–	–
(mg/L)	03.07.2017	0.280	5.720	7.580	9.110	11.140	10.870	10.240	10.370	10.510	9.690	3.300	3.440	9.670	29.000
	06.09.2017	0.120	7.335	3.610	6.720	6.975	5.235	5.250	4.570	2.968	ND	ND	ND	2.792	1.728
	09.19.2017	0.173	6.695	6.950	6.475	6.250	6.395	6.325	5.000	4.190	2.925	0.034	0.069	3.090	0.131

Notes: “–” represent no sample was collected at this point; WQ, water quality; ND, not detected



Fig. S1 Landscape of the research region: (a) recharge point, (b) the site of DQ, (c) the intersection of rivers, (d) recreation park in the middle of the river

References

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