Supporting Information

Antibiotic Pollution in Marine Food Webs in Laizhou Bay, North China: Trophodynamics and Human Exposure Implication

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Chemicals and Reagents. Sulfadiazine (SDZ), sulfathiazole (ST), sulfapyridine (SPD), sulfamerazine (SMZ), sulfadimidine (SDM), sulfamethoxazole (SMX), sulfadimethoxine (SM2), sulfamonomethoxine (SMM), sulfachlorpyridazine (SCP), trimethoprim (TMP), enoxacin (ENX), ofloxacin (OFL), ciprofloxacin (CIP), norfloxacin (NOR), enrofloxacin (ENR), roxithromycin (ROX), and anhydroerythromycin (ERY-H_2O) were purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany). Azithromycin (AZI) was obtained from Fluorochem Co., Ltd (Derbyshire, UK). Clarithromycin (CLA) were provided by TCI Co., Ltd (Shanghai, China). Stable isotope-labeled compounds, including sulfamethoxazole-D_4, atrazine-D_5, trimethoprim-D_3, ciprofloxacin-D_8, and caffeine-$^{15}N_2$, were purchased from Dr. Ehrenstorfer GmbH. Stock solutions of 1 mg/mL were prepared in methanol and stored at -4°C for 6 months.

Methanol and acetonitrile were of HPLC grade and provided by Tedia Inc. (Fairfield, Ohio, USA). Ammonium formate (analytical purity) was obtained from Sinopharm Chemical Reagent Co., Ltd. (Beijing, China). Formic acid (analytical purity) was purchased from Kermel Chemical Reagent Co., Ltd. (Tianjin, China). Ammonium hydroxide (guaranteed purity) was provided by Guangfu Fine Chemical Research Institute (Tianjin, China). Diatomite was obtained from Dionex Co., Ltd. (Sunnyvale, California, USA) and combusted at 450 ℃ for 3 hours. Ultrapure water (18.2 MΩ·cm, 25 ℃) was obtained with an OKPURE water system (Laikie Instrument Co., Ltd., Shanghai, China).

Sample Extraction and Cleanup. Approximately 1 g dry weight of a pooled biota sample was spiked with surrogate standards (10 ng each of sulfamethoxazole-D_4, atrazine-D_5, trimethoprim-D_3, and ciprofloxacin-D_8) before being thoroughly mixed with 3 g of diatomite. The mixture was placed into a 10 mL stainless steel extraction cell and extracted by pressurized liquid extraction (ASE 350, Dionex, Sunnyvale, California, USA) using methanol as extraction solvent. The extraction conditions were as follows: extraction temperature, 80 °C; extraction pressure, 10.48 MPa; preheating period, 5 min; static extraction, 5 min; flush volume 60% of the cell volume; nitrogen purge, 120 s; and number of extraction cycles, 2.

After extraction, approximately 21.5 mL of extract were transferred to a 50 mL round-bottom flask and concentrated to near dryness at 40 °C using a RE-2000 rotary evaporator (Yarong, Shanghai, China). The extract was diluted immediately with 50 mL of ultrapure water. The Oasis HLB cartridges (60 mg, 3 mL; 30 μm) were pre-conditioned with 5 mL of methanol and equilibrated with 2 mL of ultrapure water. After loading the samples, cartridges were washed with 12 mL of ultrapure water to remove matrix interferences and dried under vacuum for 30 min. The analytes and surrogate standards retained on the cartridges were eluted with 12 mL of methanol containing 5% ammonium hydroxide. The eluent was evaporated to near dryness at 40 °C and the residue was redissolved in 500 μL of the initial mobile phase (95% phase A: 5% phase B). The internal standard (10 ng of caffeine-$^{15}N_2$) was added to each sample. Samples were briefly vortexed, and filtered using a 0.22-μm syringe filter. Ten μL of extracts were injected for LC/MS/MS analysis.

Stable Isotope Analysis. An aliquot of approximately 1.50 mg of freeze-dried and homogenized samples were combusted in Sn capsules at 1020 °C. NO_x was reduced at 650 °C, the mixed gas (N_2 and CO_2) was separated and stable-carbon and stable-nitrogen isotope ratios were determined using an Isoprime 100 isotope ratio mass spectrometer interfaced to a vario PYRO cube elemental analyzer (Elementar, Hanau, Germany). The carbon stable-isotope ratio ($\delta^{13}C$) and nitrogen stable-isotope ratio ($\delta^{15}N$) of the samples are given in parts per thousand according to the formulas:

$$\delta^{15}N = \left(\frac{^{15}N/^{14}N_{sample}}{^{15}N/^{14}N_{atmosphere}} - 1 \right) \times 1000\,(\%) \qquad \text{(Eq. S1)}$$

and

$$\delta^{13}C = \left(\frac{^{13}C/^{12}C_{sample}}{^{13}C/^{12}C_{PDB}} - 1 \right) \times 1000\,(\%) \qquad \text{(Eq. S2)}$$

where the $^{15}N/^{14}N_{amosphere}$ standard value is based on atmospheric nitrogen (air) and the $^{13}C/^{12}C_{PDB}$ standard value is based on PDB (Peedee Belemnite carbonate). Triplicate analyses of two international reference materials (IAEA-600 and USGS-43) indicate an accuracy of ± 0.15‰ and ± 0.2‰ for stable-carbon and stable-nitrogen isotope measurements, respectively.

Estimation of Trophic Magnification Factors (*TMF*). *TMF* values were determined from the slope of a regression between log-transformed antibiotic concentrations (ww) in biota and the *TL*s of the sampled biota (Eq. S3 and Eq. S4). *TMF* value of each antibiotic is shown in Table S9. A *TMF* > 1 indicates biomagnification through the food web or from prey-to-predator; otherwise, trophic dilution is suggested.

$$\log[chemical\ concentration]_{ww} = a + b \cdot TL \qquad \text{(Eq. S3)}$$

$$TMF = 10^{b} \qquad \text{(Eq. S4)}$$

Determination of Relative Carbon Sources. Like δ^{15}N, δ^{13}C can be enriched through *TL*s. To eliminate the trophic influence of consumers on δ^{13}C values, the relative contribution of benthic *vs.* pelagic carbon source (relative carbon source, Figure 2) was calculated using the following equation:[1]

$$Relative\ Carbon\ Source = 1 - \frac{\delta^{13}C_{Acetes\ chinensis} - \delta^{13}C_{consumer} + \Delta\delta^{13}C(TL_{consumer} - TL_{Acetes\ chinensis})}{\delta^{13}C_{Acetes\ chinensis} - \delta^{13}C_{Rapana\ venosa}} \quad \text{(Eq. S5)}$$

where the northern maoxia shrimp (*Acetes chinensis*) is used as the pelagic source, the veined rapa whelk (*Rapana venosa*) represents the benthic source, and $\Delta\delta^{13}$C is a constant trophic enrichment factor of 1.3‰ for consumers.

Determination of Estimated Daily Intakes (*EDI*). *EDI* values for the individual antibiotics (Table S10) were calculated using the following equation and assume that 100% of the ingested antibiotics are absorbed:

$$EDI = \frac{PR \times C}{BW} \quad \text{(Eq. S6)}$$

where *EDI* is the estimated daily intake of an antibiotic in ng/kg body weight (bw)/day; *PR* is the annual per capita fish consumption of 18.63 g/person/day for rural residents and 34.45 g/person/day for urban residents in China;[2] *BW* is the average body weight of 60 kg for an adult;[3] and *C* is the 90th percentile concentration of each antibiotic (ng/g ww) detected in the present study (Table S10). The 90th percentile concentrations of FQs and MLs used to calculate *EDI* values were based on the concentrations detected in all samples collected from Laizhou Bay, China. 1/2MLOD was used to calculate the *EDI* values for antibiotic levels below the MLOD. To account for the biomagnification of SAs and trimethoprim, concentration used for calculation of the *EDI* value of each antibiotic were estimated as follows:

$$C_{\text{top consumer}} = 10^{\left[\log C_{Acetes\ chinensis} + (\log TMF) \times (TL_{\text{top consumer}} - TL_{Acetes\ chinensis})\right]} \quad \text{(Eq. S7)}$$

where $C_{\text{top consumer}}$ is the concentration of a SA or trimethoprim in the top consumer (there are no more than 4 *TL*s in a coastal food web, *i.e.*, $TL_{\text{top consumer}} = 4$); $C_{Acetes\ chinensis}$ is the 90th percentile concentration of a SA or trimethoprim in the northern maoxia shrimp (*Acetes chinensis*) ($TL_{Acetes\ chinensis} = 2$); and *TMF* is the trophic magnification factor of the antibiotics estimated in the present study.

Determination of Hazard Quotients (*HQ*). Human health risks of rural and urban residents associated with antibiotic exposure following the consumption of seafood were assessed using hazard quotients calculated for each antibiotic with the following equation:

$$HQ = \frac{EDI}{ADI} \quad \text{(Eq. S8)}$$

where *HQ* is the hazard quotient and *ADI* is the acceptable daily intake (μg/kg bw/day) of an antibiotic. *ADI* values are based on a review of the data published by the World Health Organization/Food and Agriculture Organization (WHO/FAO), the Joint Expert Committee on Food Additives (JECFA), the Australian Government, Department of Health, Office of Chemical Safety (OCS), or other relevant sources. *ADI*s and *HQ* values of all 19 antibiotics are summarized in Table S10. A comparison of *HQ* values of individual antibiotics for rural *versus* urban residents is shown in Figure S4.

Table S1. Physicochemical properties of antibiotics investigated in this study.

Antibiotics	Abbreviation	CAS number	logK_{OW} [a]	log$D$ [b]	pK_a [a]	f_n [b]	Predominant ionized species at pH = 7.5
Macrolides (MLs)							
Azithromycin	AZI	83905-01-5	4.02	2.76	8.74	0.05	Cation
Clarithromycin	CLA	81103-11-9	3.16	1.66	8.99	0.03	Cation
Anhydroerythromycin	ERY-H_2O	114-07-8	3.06	1.64	8.90	0.04	Cation
Roxithromycin	ROX	80214-83-1	2.75 [j]	2.00	8.16 [c]	0.18	Cation
Sulfonamides (SAs)							
Sulfadiazine	SDZ	68-35-9	0.39 [d]	-0.24	6.99 [d]	0.24	Anion
Sulfachlorpyridazine	SCP	80-32-0	1.30 [e]	0.26	6.50 [f]	0.09	Anion
Sulfamonomethoxine	SMM	1220-83-3	0.61 [c]	-0.28	6.67 [c]	0.13	Anion
Sulfadimethoxine	SM2	122-11-2	1.63 [j]	-0.02	5.86 [g]	0.02	Anion
Sulfamethoxazole	SMX	723-46-6	0.89	-0.24	6.40 [h]	0.07	Anion
Sulfapyridine	SPD	144-83-2	0.03 [c]	-0.01	8.54 [c]	0.92	Anion
Sulfathiazole	ST	72-14-0	0.05	-0.40	7.24	0.35	Anion
Sulfamerazine	SMZ	127-79-7	0.38 [c]	0.00	7.35 [c]	0.41	Anion
Sulfadimidine	SDM	57-68-1	0.14	-0.09	7.65	0.59	Anion
Trimethoprim	TMP	738-70-5	0.79 [c]	0.09	6.90 [c]	0.20	Anion

Continued Table S1.

Antibiotics	Abbreviation	CAS number	logK_{OW} [a]	log$D$ [b]	pK_a [a]	f_n [b]	Predominant ionized species at pH = 7.5
Fluoroquinolones (FQs)							
Norfloxacin	NOR	70458-96-7	0.46	-0.81	8.75	0.05	Cation
Enoxacin	ENX	74011-58-8	-0.20 [c]	-0.97	8.19 [c]	0.17	Cation
Ofloxacin	OFL	82419-36-1	-0.39	-0.87	7.81 [i]	0.33	Cation
Enrofloxacin	ENR	93106-60-6	0.44 [c]	-0.79	8.70 [j]	0.06	Cation
Ciprofloxacin	CIP	85721-33-1	0.28	-0.98	8.74	0.05	Cation

[a] Values were obtained from the Hazardous Substances Data Bank (HSDB) (available at https://toxnet.nlm.nih.gov/newtoxnet/hsdb.htm) unless indicated otherwise.

[b] pH dependent distribution coefficient (log$D$) and fraction of neutral molecules (f_n) were calculated according to reference (4) at pH = 7.5, which is suggested to be the mean value of pH ranges in the intestinal tract of marine fish by reference (5).

[c] Values were calculated using Advanced Chemistry Development (ACD/I-Lab) Software 2.0 (available at https://ilab.acdlabs.com/iLab2/). All pK_a values are given for predominant ionized species at pH = 7.5.

[d] From reference (6). [e] From reference (7). [f] From reference (8). [g] From reference (9). [h] From reference (10). [i] From reference (11). [j] From reference (12). [j] From ChemID*plus* (available at https://chem.nlm.nih.gov/chemidplus/chemidlite.jsp)

Table S2. Summary of the average body lengths and body weights of the specimens collected from Laizhou Bay, China, and the corresponding sampling locations (Figure S1) and sampling times.

Species [a]	Sampling location [b]	Sampling time	Length (cm) [c]	Weight (g) [c]
Invertebrates (9)				
Veined rapa whelk (*Rapana venosa*)	S2, S4, S5	2014/10, 2015/4	8.2 ± 0.5	96 ± 6
Mactra quadrangularis (*Mactra veneriformis*)	S1	2014/6	3.3 ± 0.2	8.4 ± 1.6
Razor clam (*Sinonovacula constricta*)	S3	2014/6	6.7 ± 0.4	8.5 ± 1.8
Octopus (*Octopus ocellatus*)	S2, S4	2014/10, 2015/4	27.0 ± 0.2	90 ± 7
Mantis shrimp (*Oratosquilla oratoria*)	S2, S4	2014/10	13.7 ± 1.2	32 ± 4
Northern maoxia shrimp (*Acetes chinenisis*)	S2, S5	2014/10, 2015/4	3.3 ± 1.6	1.7 ± 1.0
Japanese stone crab (*Charybdis japonica*)	S2, S4	2014/10, 2015/4	7.6 ± 0.4	36 ± 6
Swimming crab (*Portunus trituberculatus*)	S2, S4	2014/10, 2015/4	10.3 ± 1.1	52 ± 10
Prawn (*Fenneropenaeus chinensis*)	S2	2014/10, 2015/4	6.7 ± 1.4	8.5 ± 1.8
Benthic fish (5)				
Javeline goby (*Acanthogobius hasta*)	S1, S2	2014/3, 2014/6, 2014/10, 2015/4	27.4 ± 2.7	80 ± 8
Eelgoby (*Odontamblyopus rubicundus*)	S4	2014/6	30.7 ± 2.8	62 ± 6
Fat greenling (*Hexagrammos otakii*)	S4	2014/6	21.5 ± 0.9	121 ± 7
Flathead (*Platycephalus indlcus*)	S1, S2, S4	2014/3, 2014/10	42.8 ± 3.7	430 ± 30
Tongue sole (*Cymoglossus robustus*)	S2, S4	2014/10, 2015/4	31.0 ± 2.1	176 ± 51

Continued Table S2.

Species [a]	Sampling locations [b]	Sampling time	Length (cm) [c]	Weight (g) [c]
Pelagic fish (5)				
Halfbeak (*Hemirhamphus sajori*)	S4	2014/10	10.7 ± 1.0	1.9 ± 0.7
Dotted gizzard shad (*Konosirus punctatus*)	S2	2015/4	18.1 ± 1.7	65 ± 9
Mullet (*Liza haematocheilus*)	S2	2014/3, 2014/6, 2014/10	25.4 ± 1.9	152 ± 37
Chinese sea perch (*Lateolabrax maculatus*)	S2	2014/10, 2015/4	24.8 ± 2.6	156 ± 38
Silvery pomfret (*Pampus argenteus*)	S5	2014/10	16.5± 1.2	134 ± 15

[a] The species were identified and classified using the SealifeBase database (available at http://www.sealifebase.org) and Checklist of Marine biota of China Seas.[13]

[b] The coordinates of longitude and latitude for sampling sites are showed in Figure S1.

[c] Data are presented as the mean value ± one standard deviation.

Table S3. Optimized LC-MS/MS parameters and retention times of the antibiotics analyzed in the present study.

Compound [a]	MRM transition (*m/z*)	Fragment voltage (V)	Collision energy (eV)	Retention time (min)
AZI	749.9 → 115.9	215	49	14.7
ERY-H_2O	716.5 → 158.2	160	25	20.1
CLA	748.9 → 158.0	170	29	21.1
ROX	838.0 → 158.0	190	37	21.5
SDZ	251.3 → 92.1	100	25	5.0
SCP	285.0 → 155.8	110	13	10.0
SMM	281.3 → 92.1	120	36	10.3
SM2	311.0 → 155.9	125	17	13.9
SMX	254.0 → 108.0	105	25	10.7
SPD	250.3 → 155.9	110	13	6.7
ST	256.0 → 155.9	110	13	6.3
SMZ	265.0 → 108.0	122	29	7.1
SDM	279.0 → 92.0	112	33	8.8
TMP	291.0 → 230.0	165	21	8.5
ENX	321.3 → 303.2	130	21	9.2
CIP	332.2 → 314.1	124	17	9.8
ENR	360.4 → 342.1	134	17	10.7

Continued Table S3.

Compound [a]	MRM transition (*m/z*)	Fragment voltage (V)	Collision energy (eV)	Retention time (min)
OFL	362.0 → 318.0	130	15	9.5
NOR	320.0 → 302.0	128	21	9.6
Sulfamethoxazole-D_4 (surrogate)	258.0 → 112.0	105	25	10.7
Atrazine-D_5 (surrogate)	221.5 → 179.4	80	12	17.9
Trimethoprim-D_3 (surrogate)	294.4 → 123.0	152	21	8.5
Ciprofloxacin-D_8 (surrogate)	340.2 → 235.1	125	44	9.9
Caffeine-$^{15}N_2$ (IS)	197.1 → 139.1	90	18	7.5

[a] The full names of the compounds can be found in Table S1.

Table S4. Surrogate standards, linear range of matrix matched calibration curves, method detection limits (MLOD), method quantification limits (MLOQ), spiked average recoveries and relative standard deviations (RSD, $n = 6$) for each antibiotic.

Compound [a]	Spiked surrogate	Range of calibration curve (ng/mL)	r	MLOD [b] (ng/g ww)	MLOQ [b] (ng/g ww)	Recovery [c] (%)	RSD (%)
CIP	Ciprofloxacin-D_8	2 - 2000	0.998	1.5	4.9	98	14
ENX	Ciprofloxacin-D_8	2 - 2000	0.998	1.0	3.5	49	18
ENR	Ciprofloxacin-D_8	2 - 2000	0.998	1.1	3.6	97	13
NOR	Ciprofloxacin-D_8	2 - 2000	0.997	1.2	3.9	74	13
OFL	Ciprofloxacin-D_8	2 - 2000	0.998	0.7	2.2	128	13
AZI	Atrazine-D_5	0.5 - 100	0.997	0.3	0.9	111	9
CLA	Atrazine-D_5	0.5 - 100	0.997	0.3	1.0	91	15
ERY-H_2O	Atrazine-D_5	0.5 - 100	0.995	0.1	0.3	82	11
ROX	Atrazine-D_5	0.5 - 100	0.998	0.1	0.4	64	14
TMP	Trimethoprim-D_3	0.1 - 100	0.998	0.02	0.08	90	14
SMX	Sulfamethoxazole-D_4	1 - 1000	0.999	0.9	2.8	117	12
SM2	Sulfamethoxazole-D_4	1 - 1000	0.996	0.2	0.7	80	18
SPD	Sulfamethoxazole-D_4	1 - 1000	0.997	0.3	0.9	113	16

Continued Table S4.

Compound [a]	Spiked surrogate	Range of calibration curve (ng/mL)	*r*	MLOD [b] (ng/g ww)	MLOQ [b] (ng/g ww)	Recovery [c] (%)	RSD (%)
ST	Sulfamethoxazole-D4	1 - 1000	0.996	0.5	1.8	67	17
SDM	Sulfamethoxazole-D4	1 - 1000	0.998	0.6	2.0	111	19
SMZ	Sulfamethoxazole-D4	1 - 1000	0.997	0.4	1.2	112	17
SDZ	Sulfamethoxazole-D4	1 - 1000	0.997	0.5	1.7	110	14
SCP	Sulfamethoxazole-D4	1 - 1000	0.997	0.4	1.2	102	18
SMM	Sulfamethoxazole-D4	1 - 1000	0.997	1.5	5.0	114	8

[a] The full names of the compounds can be found in Table S1.

[b] MLOD and MLOQ are defined as three and ten times the standard deviation (SD) of the mean procedure blanks (n = 6), respectively.

[c] The recovery of each antibiotic was determined by analysis of six replicates of non-contaminated biotic matrix (*i.e.*, farm raised fish) spiked with a mixture of antibiotic standards. Different concentrations (5 to 250 ng/g) were spiked for each antibiotic to account for difference in response of all antibiotics during simultaneous analysis.

Table S5. Detection frequencies of the target antibiotics in all samples.

Compound [a]	Detection frequency (%)	Compound [a]	Detection frequency (%)
SCP	63	CIP	23
SDZ	47	ENX	33
SM2	23	ENR	60
SMZ	72	NOR	49
SDM	47	OFL	67
SMX	95	AZI	21
SMM	39	CLA	25
SPD	25	ERY-H_2O	58
ST	47	ROX	23
TMP	91		

[a] The full names of the compounds can be found in Table S1.

Table S6. Moisture content (%), estimated trophic level (*TL*), mean concentrations in ng/g ww (ng/g dw) of sulfonamide, fluoroquinolone, macrolide antibiotics and trimethoprim, and detection frequency (%) in different species collected from Laizhou Bay, China.

Species	*n* [a]	Moisture content [b]	*TL* [c]	∑SAs [d]	TMP [d]	∑FQs [d]	∑MLs [d]	Total conc. [d]	Frequency
Mantis shrimp	16	76	3.60 ± 0.04	190 ± 10 (36 ± 2) [e]	1.9 ± 0.1 (0.35 ± 0.02)	200 ± 20 (37 ± 3)	1.8 ± 0.1 (0.34 ± 0.03)	390 ± 20 (74 ± 3)	47
Swimming crab	8	72	3.40 ± 0.10	220 ± 50 (57 ± 13)	1.1 ± 0.5 (0.3 ± 0.1)	310 ± 10 (81 ± 3)	4.5 ± 1.0 (1.2 ± 0.3)	540 ± 60 (140 ± 20)	90
Mactra quadrangularis	34	73	2.60 ± 0.04	110 ± 5 (19 ± 1)	17 ± 2 (2.8 ± 0.3)	1,200 ± 90 (190 ± 10)	3.5 ± 0.4 (0.6 ± 0.1)	1,300 ± 90 (220 ± 10)	79
Razor clam	11	81	2.50 ± 0.05	21 ± 2 (4.1 ± 0.3)	34 ± 20 (6.6 ± 4.0)	450 ± 40 (88 ± 8)	nd	500 ± 50 (98 ± 9)	47
Japanese stone crab	13	79	3.40 ± 0.10	170 ± 13 (35 ± 3)	4.0 ± 3.0 (0.8 ± 0.7)	160 ± 40 (33 ± 8)	3.9 ± 4.0 (0.8 ± 0.7)	340 ± 30 (70 ± 6)	74
Veined rapa whelk	15	77	2.30 ± 0.06	82 ± 7 (22 ± 2)	8.9 ± 2.0 (2.4 ± 0.5)	330 ± 40 (90 ± 10)	0.7 ± 1.0 (0.2 ± 0.3)	420 ± 40 (110 ± 10)	47
Northern maoxia shrimp	49	74	2.00 ± 0.10	51 ± 6 (12 ± 2)	0.30 ± 0.02 (0.07 ± 0.01)	nd [f]	13 ± 3 (2.9 ± 0.8)	64 ± 10 (15 ± 2)	53
Prawn	11	81	3.20 ± 0.30	77 ± 30 (22 ± 7)	1.6 ± 0.8 (0.2 ± 0.3)	190 ± 30 (54 ± 9)	3.7 ± 1.0 (1.1 ± 0.3)	270 ± 40 (77 ± 10)	47
Octopus	3	83	3.50 ± 0.06	350 ± 60 (83 ± 14)	0.5 ± 0.2 (0.12 ± 0.04)	68 ± 4 (16 ± 1)	4.4 ± 2.0 (1.1 ± 0.5)	420 ± 50 (100 ± 10)	79

Continued Table S6.

Species	*n* [a]	Moisture content [b]	*TL* [c]	∑SAs [d]	TMP [d]	∑FQ [d]	∑MLs [d]	Total conc. [d]	Frequency
Fat greenling	6	41	3.50 ± 0.02	290 ± 30 (69 ± 6)	2.4 [g] 0.6 [g]	92 ± 5 (22 ± 1)	nd [f]	380 ± 20 (91 ± 6)	21
Javeline goby	17	73	4.00 ± 0.03	110 ± 30 (22 ± 7)	2.0 ± 2.0 (0.4 ± 0.5)	100 ± 2 (21 ± 0.4)	0.2 ± 0.3 (0.05 ± 0.07)	210 ± 40 (44 ± 8)	42
Flathead	3	79	3.90 ± 0.06	340 ± 40 (70 ± 9)	2.0 ± 2.0 (0.4 ± 0.4)	48 ± 30 (10 ± 7)	1.4 ± 1.0 (0.3 ± 0.2)	390 ± 60 (81 ± 10)	37
Eelgoby	7	78	3.50 ± 0.01	440 ± 10 (100 ± 2)	2.2 ± 0.2 (0.51 ± 0.04)	130 ± 30 (30 ± 8)	nd	580 ± 40 (130 ± 10)	42
Tongue sole	15	77	3.30 ± 0.30	110 ± 70 (25 ± 16)	5.2 ± 3.0 (1.2 ± 0.7)	94 ± 20 (21 ± 4)	1.2 ± 1.0 (0.3 ± 0.3)	210 ± 50 (47 ± 10)	47
Chinese sea perch	17	79	3.90 ± 0.30	310 ± 140 (74 ± 30)	5.4 ± 4.0 (1.3 ± 1.0)	16 ± 10 (3.8 ± 2.0)	7.5 ± 4.0 (1.8 ± 1.0)	340 ± 150 (81 ± 30)	37
Mullet	11	76	3.20 ± 0.20	100 ± 9 (22 ± 2)	1.3 ± 1.0 (0.3 ± 0.2)	12 ± 7 (3 ± 2)	0.4 ± 0.6 (0.1 ± 0.1)	110 ± 20 (26 ± 4)	32
Silvery pomfret	6	69	4.10 ± 0.02	150 ± 20 (46 ± 5)	6.4 ± 0.3 (2.0 ± 0.1)	84 ± 30 (26 ± 8)	3.2 ± 1.0 (1.0 ± 0.4)	240 ± 20 (75 ± 6)	68
Dotted gizzard shad	6	78	3.30 ± 0.03	100 ± 10 (62 ± 7)	20 ± 20 (7.7 ± 10.0)	93 ± 10 (55 ± 6)	4.2 ± 2.0 (2.5 ± 1.0)	210 ± 30 (130 ± 20)	26
Halfbeak	32	76	2.20 ± 0.10	52 ± 20 (14 ± 6)	5.0 ± 1.0 (1.3 ± 0.3)	86 ± 20 (23 ± 5)	5.7 ± 1.0 (1.5 ± 0.3)	150 ± 40 (40 ± 10)	68

[a] *n* is the number of organisms sampled for each species, and three different homogenized samples were analyzed for each species.

[b] Water content was calculated based on the difference between wet and dry weights.

[c] *TL* values were calculated using equation (2) as described in data analysis of the text and presented as the mean value ± one standard deviation.

[d] ∑SAs: sum of sulfonamide antibiotics; TMP: trimethoprim; ∑FQs: sum of fluoroquinolone antibiotics; ∑MLs: sum of macrolide antibiotics; Total conc.: sum of all antibiotics investigated in this study. All concentrations are presented as the mean value ± one standard deviation.

[e] Concentrations on a dry weight basis for each species are given in parentheses.

[f] nd: value below MLOD.

[g] Analyte was detected in only one pooled sample.

Table S7. Comparison of antibiotic levels detected in aquatic animals in areas around the world.

Study region	Collected species [a]	Antibiotic class [b]	Conc. Range (ng/g ww)	Reference
Laizhou Bay, North China	Fish and invertebrates (n = 280)	∑SAs + TMP	nd [e] - 453 (170 [c])	This study
		∑FQs	nd - 1,200 (190)	
		∑MLs	nd - 15 (2.9)	
Coastal areas, Hailing Island, South China	Shrimps, crabs, mollusks, and fish (n = 39)	∑SAs + TMP	nd - 58.6	(14)
		∑FQs	nd - 153.2	
		∑MLs	nd - 15,093	
Coastal areas, Dalian, North China	*Crassostrea gigas and scallops* (n = 13)	∑SAs	2.18 - 63.87	(15)
		∑CAPs	nd - 4.03	
Bohai Sea, North China	Mollusks (n = 190)	∑SAs	nd - 76.7(6.0)[d]	(16)
		∑FQs	0.7 - 1,575.1(86.8) [d]	
		∑MLs	nd - 36.2(2.6) [d]	
Baiyangdian Lake, North China	Crustacean and fish (n =23)	∑SAs	nd - 98.3 [d]	(17)
		∑FQs	17.8 - 167 [d]	
		∑MLs	nd - 182 [d]	
Rivers, Pearl River Delta, China	Fish (n = 128)	∑SAs	nd - 9.3	(18)
		∑FQs	nd -19.0	
		∑MLs	nd - 2.36 (< 0.49)	

Continued Table S7.

Study region	Collected species [a]	Antibiotic class [b]	Conc. Ranges (ng/g ww)	Reference
Haihe River, North China	Fish (n = 10)	∑SAs	nd - 996 [d]	(19)
		∑FQs	nd - 63.5 [d]	
		∑MLs	nd - 45.1 (7.8) [d]	
Aquatic markets, Canada	Shrimps and fish (n = 30)	∑FQs	nd - 0.73	(20)
		∑CAPs	nd - 0.4	
Rivers, USA	Fish (n = 144)	SMX + TMP	nd	(21)
Rivers, Germany	Bream (n = 20)	SMX + TMP	nd	(22)
Coastal areas, Ireland	Mussels (n = 45)	TMP	nd - 9.22 [d]	(23)

[a] Numbers in parenthesis are the number of analyzed samples.

[b] ∑SAs: sum of sulfonamide antibiotics; SMX: sulfamethoxazole; TMP: trimethoprim; ∑FQs: sum of fluoroquinolone antibiotics; ∑MLs: sum of macrolide antibiotics; ∑CAPs: sum of chloramphenicol antibiotics.

[c] Values in parentheses are the mean concentrations.

[d] Concentrations are reported by ng/g dry weight.

[e] nd: value below MLOD.

Table S8. Analysis of variance (ANOVA) of the calculated trophic level (*TL*) values at different sampling times (*i.e.*, June 2014, October 2014 and April 2015) by SPSS 19.0.[a]

	Sum of Squares	D*f*	Mean Square	F	Sig.
Between Groups	0.313	2	0.157	0.394	0.678
Within Groups	11.916	30	0.397		
Total	12.230	32			

[a] *TL* values of a species derived from isotope determination with at least two different sampling times are used in this analysis.

Table S9. Slope, correlation coefficients (*r*) and *p*-values of regression analyses between logarithm concentrations and trophic levels (see Figure 3), and *TMF*s and 95% confidence intervals (CIs) of target antibiotics and ΣSAs, ΣFQs, ΣMLs.

Antibiotic [a]	slope	*r*	*p*-value	*TMF* [c]	95% CI
SCP	0.52	0.93	**< 0.001** [b]	3.3	2.5 - 4.5
SDZ	0.57	0.83	**0.003**	3.7	1.8 - 7.6
SM2	0.14	- [e]	0.624	1.4	0.2 - 9.2
SMZ	0.45	-	0.368	2.8	0.3 - 30.4
SDM	0.59	0.77	**0.016**	3.9	1.4 - 10.7
SMX	0.34	0.85	**< 0.001**	2.2	1.7 - 2.9
SMM	0.15	0.65	**0.036**	1.4	1.0 - 1.9
SPD	0.09	0.80	0.065	1.2	1.0 - 1.6
ST	0.09	0.54	0.061	1.2	1.0 - 1.5
∑SAs [d]	0.42	0.78	**< 0.001**	2.6	1.7 - 4.0
TMP	0.38	0.54	**0.019**	2.4	1.2 - 4.8
CIP	-0.36	-	0.459	0.4	0.0 - 9.5
ENX	-0.28	0.66	**0.044**	0.5	0.3 - 1.0
ENR	-0.40	0.76	**0.003**	0.4	0.2 - 0.7
NOR	-0.09	-	0.481	0.8	0.4 - 1.6

Continued Table S9.

Antibiotic [a]	slope	*r*	*p*-value	*TMF* [c]	95% CI
OFL	-0.42	0.77	**0.001** [b]	0.4	0.2 - 0.6
∑FQs [d]	-0.48	0.69	**0.002**	0.3	0.2 - 0.6
AZI	-0.16	0.88	**0.031**	0.7	0.5 - 0.9
CLA	-0.02	-	0.900	1.0	0.3 - 2.6
ERY-H_2O	-0.20	0.66	**0.013**	0.6	0.5 - 0.9
ROX	- [e]	-	-	-	-
∑MLs [d]	-0.32	0.81	**0.001**	0.5	0.3 - 0.7
Total [d]	-0.14	0.22	0.205	0.7	0.4 - 1.2

[a] The full names of the compounds can be found in Table S1.

[b] *p*-values in bold print represent statistically significant increases or decreases of the wet weight concentration (*i. e.*, < 0.05).

[c] *TMF* values were calculated using Eq. S3 and Eq. S4 as described above.

[d] ∑SAs: sum of sulfonamide antibiotics; ∑FQs: sum of fluoroquinolone antibiotics; ∑MLs: sum of macrolide antibiotics; Total: sum of all antibiotics investigated in this study.

[e] -: not available.

Table S10. 90th percentile antibiotic concentrations (*C*), estimation daily intake (*EDI*) values and associated hazard quotients (*HQ*) of antibiotics for rural and urban residents and acceptable daily intake (*ADI*) values obtained from the literature.

Antibiotic [a]	*C* [b] (ng/g ww)	*EDI* [c] (ng/kg bw/d)		*HQ* [d] × 10^2		*ADI* (μg/kg bw/d)	Reference
		urban	rural	urban	rural		
SCP	39.8	22.9	12.4	-	-	- [e]	-
SDZ	3.5	2.0	1.1	0.01	0.005	20	(24)
SM2	1.8	1.1	0.6	0.01	0.006	10	(25)
SMZ	38.1	21.9	11.8	-	-	-	-
SDM	36.0	20.7	11.2	0.04	0.02	50	(26)
SMX	160.9	92.4	50.0	0.2	0.09	57	(27)
SMM	14.3	8.2	4.4	0.1	0.07	6.0	(24)
SPD	1.9	1.1	0.6	0.03	0.02	3.3	(27)
ST	4.0	2.3	1.3	0.005	0.003	50	(28)
TMP	1.8	1.0	0.6	0.02	0.01	4.0	(28)
∑SAs [e]	-	-	-	0.4	0.2	-	-
CIP	27.8	16.0	8.6	1.0	0.5	1.6	(29)
ENX	32.0	18.4	9.9	-	-	-	-
ENR	250.9	144.1	77.9	7.2	3.9	2.0	(30)
NOR	115.9	66.6	36.0	0.6	0.3	11.4	(27)

Continued Table S10.

Antibiotic [a]	*C* [b] (ng/g ww)	*EDI* [c] (ng/kg bw/d)		*HQ* [d] × 10^2		*ADI* (μg/kg bw/d)	Reference
		urban	rural	urban	rural		
OFL	49.4	28.4	15.4	0.5	0.3	5.7	(27)
∑FQs	-	- [e]	-	9.4	5.0	-	-
AZI	1.7	1.0	0.5	0.06	0.03	1.7	(31)
CLA	3.6	2.1	1.1	1.0	0.6	0.2	(32)
ERY-H_2O	2.5	1.4	0.8	0.2	0.1	0.7	(33)
ROX	0.8	0.5	0.3	0.1	0.07	0.4	(34)
∑MLs	-	-	-	1.4	0.8	-	-

[a] The full names of the compounds can be found in Table S1.

[b] For SAs and TMP, concentration used to calculate the *EDI* value of each antibiotic (*C*) was estimated using Eq. S7 as described above. For FQs and MLs, *C* was the 90th percentile concentration of each antibiotic detected in the present study. 1/2MLOD was used to calculate the *EDI* values for antibiotic levels below the MLOD.

[c] *EDI*s were calculated using Eq. S6 as described above based on the consumption of a diet containing only seafood.

[d] *HQ* values were calculated using Eq. S8 as described above.

[e] -: not available.

[f] ∑SAs: TMP was included based on similar mode of action (MOA) between SAs and TMP.

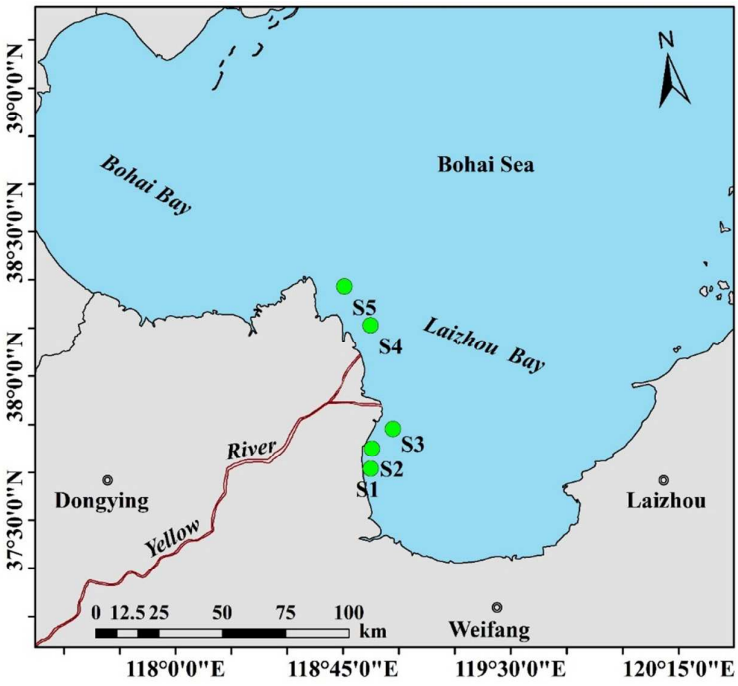

Figure S1. Sampling locations of this study.

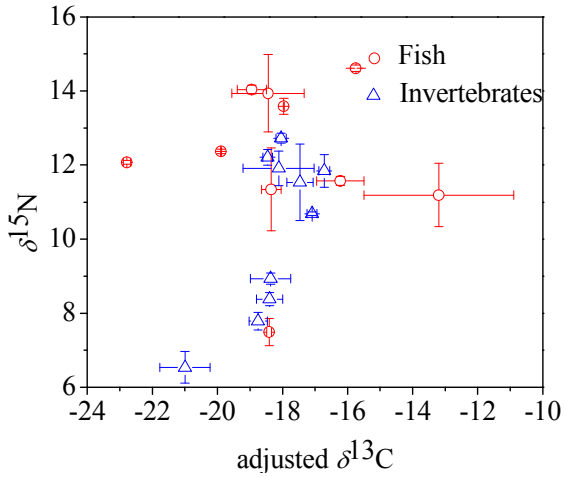

Figure S2. δ^{15}N and adjusted δ^{13}C values for the species in the coastal food web from Laizhou Bay, China. δ^{15}N and δ^{13}C values were calculated using Eq. S1 and Eq. S2, respectively, as described above. All δ^{13}C values were mathematically adjusted for the C/N ratios obtained during the determination of the stable-isotope ratios as described in equation (1).

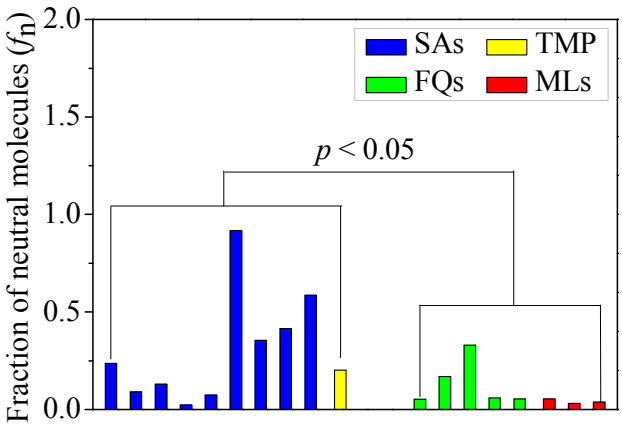

Figure S3. Significant difference ($p < 0.05$) on fraction of neutral molecules (f_n) values between SA antibiotics (including TMP) and the other two antibiotic groups (*i.e.*, FQs and MLs). f_n values of antibiotics (Table S1) were calculated using an equation described in Fu et al. at pH = 7.5, which is the mean value of pH ranges in the intestinal tract of marine fish reported by Ou et al.[4,5]

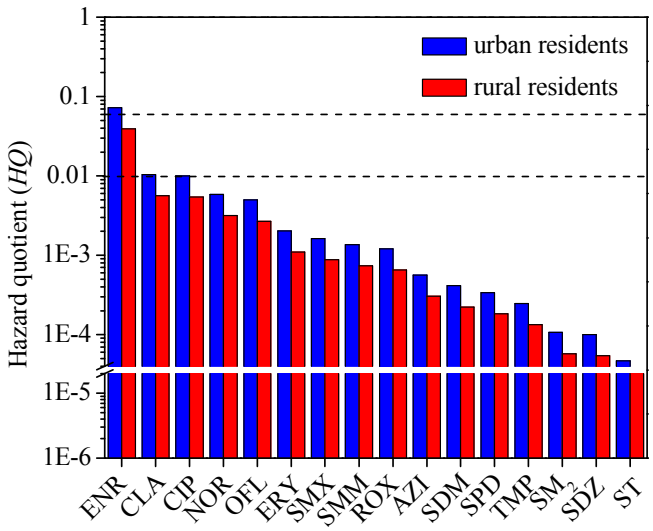

Figure S4. Hazard quotients (*HQ*) of antibiotics for rural and urban residents based on consumption of seafood only. Dotted lines represent threshold values of a considerable risk (*i.e.*, *HQ* = 0.01) and a distinct risk (*i.e.*, *HQ* = 0.05), respectively.

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