Occurrence and Transport of Tetracycline, Sulfonamide, Quinolone, and Macrolide Antibiotics in the Haihe River Basin, China

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Ⓢ Supporting Information

ABSTRACT: The occurrence and transport of 12 antibiotics (from the tetracycline, sulfonamide, quinolone, and macrolide families) was studied in a 72-km stretch of the Haihe River, China, and in six of its tributaries. Aqueous and sediment samples were analyzed by HPLC—MS/MS. Sulfonamides were detected at the highest concentrations (24—385 ng/L) and highest frequencies (76—100%). Eight of the 12 antibiotics likely originated from veterinary applications in swine farms and fishponds, and concentrations at these sources (0.12—47 μg/L) were 1—2 orders of magnitude higher than in the effluent of local wastewater treatment plants. Sulfachloropyridazine (SCP) was detected in all swine farm and fishpond samples (maximum concentration 47 μg/L), which suggests its potential usefulness to indicate livestock source pollution in the Haihe River basin. Hydrological and chemical factors that may influence antibiotic distribution in the Haihe River were considered by multiple regression analysis. River flow rate exerted the most significant effect on the first-order attenuation coefficient (K) for sulfonamides, quinolones, and macrolides, with higher flow rates resulting in higher K, probably due to dilution. For tetracyclines, sediment total organic matter and cation exchange capacity exerted a greater impact on K than flow rate, indicating that adsorption to sediments plays an important role in attenuating tetracycline migration. Overall, the predominance of sulfonamides in the Haihe River underscores the need to consider regulating their veterinary use and improving the management and treatment of associated releases.

INTRODUCTION

China leads the world in antibiotic production capacity,[1] with an annual production of about 210 000 t, of which 85% is utilized in animal agriculture and medicine.[2] A significant percentage of these antibiotics (25—75%) is excreted unaltered in feces and persists in soil after land application,[3] which represents a non-point source that increases the concentration of antibiotic residues in the environment. Antibiotics from domestic sources and pharmaceutical industries can also be transported in sewage to wastewater treatment plants (WWTPs),[4—6] where they tend to resist biodegradation[7,8] and are discharged to rivers as point sources in WWTP effluents.[9—12] Additional potential sources of antibiotics draining to aquatic ecosystem include feces from confined animal feeding operations (CAFOs),[11,13,14] which are often used as fertilizer (manure), and drainage from aquaculture ponds.[15] The increased presence of antibiotics in the environment raises concerns about their propensity to select for resistant bacteria and facilitate the establishment and amplification of pathogenic reservoirs that threaten public health.[16—18] Potential contamination of drinking water supplies by such emerging pollutants is also of concern.[19]

The types and concentrations of antibiotics in the environment vary among areas and countries, depending on antibiotic consumption and use patterns.[20] In some industrialized countries, WWTP effluents containing antibiotics used in human medicine are the major sources of antibiotics in the aquatic environment.[21] Antibiotic occurrence in aquatic systems is also affected by their chemical stability and partition characteristics.[22] For example, sulfonamides exhibit high solubility and chemical stability in water, whereas macrolides tend to be hydrolyzed or sorbed to soil and sediments;[23] quinolones are susceptible to photodegradation[24] and are also adsorbed in sediments;[25] and tetracyclines have a high affinity for soil organic matter through cation bridging and cation exchange.[26—28] To date, valuable
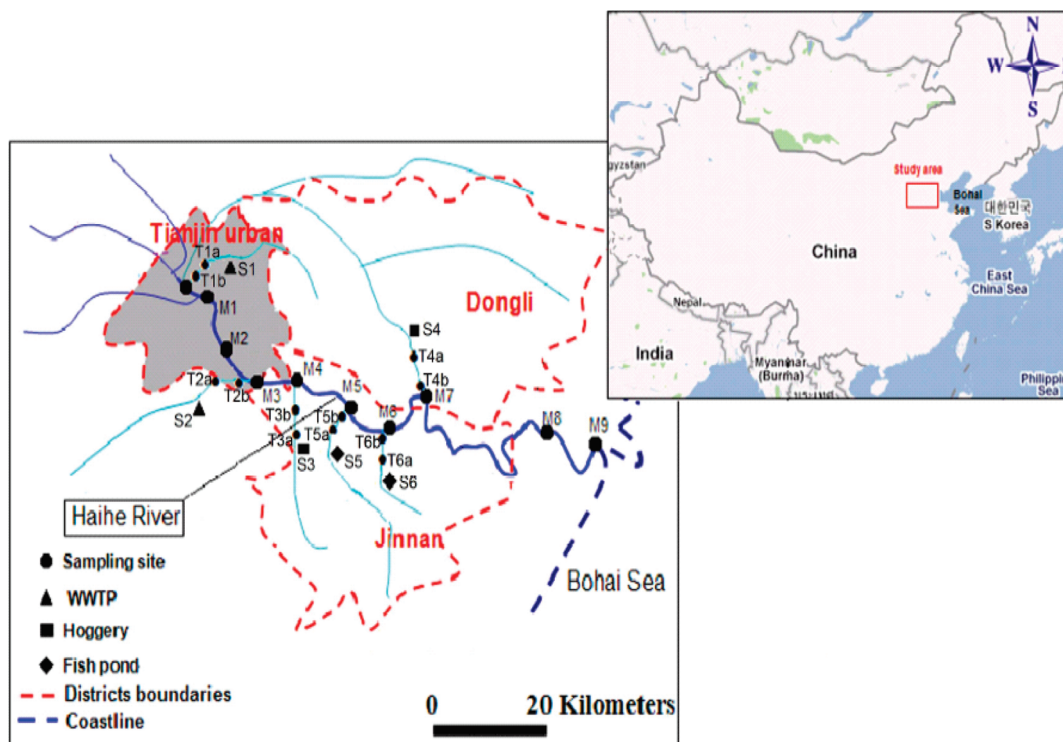
Figure 1. Sampling locations in the main stream of the Haihe River (M), tributaries (T), and potential antibiotic sources (S). Two locations (Ta, Tb) were sampled downstream from pollution sources at each tributary. Sampling locations are numbered along the river flow direction.

insight on the fate of specific antibiotics has been obtained through laboratory studies. Yet the environmental factors that determine the reach and attenuation patterns of antibiotics at the regional scale are not fully understood, and limited data exist on the spatial and temporal variability of antibiotic occurrence in the environment, which underscores the need for complementary regional studies.

The Haihe River basin (discharging into the Bohai Sea) is the largest water system in northern China and drains an area of $265\,000\ km^2$ that includes the fastest growing economic region of China as well as $120\,000\ km^2$ of farmland.[29] There are numerous inputs of antibiotics from livestock and aquaculture waste to the Haihe River. In the vicinity of the city of Tianjin (approximately 10 million inhabitants), animal husbandry and aquaculture contribute 57% of the total agricultural gross domestic product, which is twice China's national average of 30%.[30] Inputs from human sources via WWTPs (which serve 90% of Tinajin's population) have also been shown to be important sources of pharmaceutical compounds.[31] Previous studies reported the detection of oxytetracycline (OTC), tetracycline (TC), sulfadiazine (SD), ciprofloxacin (CIP), sulfachloropyridazine (SCP), and sulfamethoxazole (SM2) at $0.3-173\ mg/kg$ in livestock excrement at CAFOs along the Haihe River.[32] However, no comprehensive regional study has been conducted to characterize the frequency of occurrence, migration, and attenuation of selected antibiotics in the Haihe River basin and the relative contributions from agricultural drainages and wastewater treatment plant discharges.

This study characterizes the occurrence of tetracycline, sulfonamide, quinolone, and macrolide residues in the Haihe River and its main tributaries and assesses their sources and environmental factors that influence their migration and attenuation. The structures and chemical properties of 12 selected antibiotics are shown in the

Supporting Information (Table S1). To our knowledge, this is the first regional study to systematically characterize the distribution, migration, and attenuation of selected antibiotics in the Haihe River basin under varying hydrological conditions in light of their physicochemical properties.

■ MATERIALS AND METHODS

Sampling Sites and Sample Collection. Multiple sampling points were established to investigate the loading and potential migration of antibiotics from putative sources to the Haihe River tributaries and subsequently to the main stream (Figure 1). These include six sites on the tributaries representing potential sources (S1–S6), at least two sampling sites for each of six tributaries (T1a, T1b, T2a, T2b, and so on), and nine sites on the Haihe River (M1–M9). Sites M1–M3 are located in an urban area with high population density. The effluents from two of several WWTPs located in this area (S1 and S2) were also sampled (see Table S2 in the Supporting Information for additional information on WWTPs that discharge to Haihe River tributaries). Sites M4–M7 were located in agriculturally influenced areas (Jinnan and Dongli Districts), which included fishponds, feedlots, and dairies. Sites M8 and M9 are located near the mouth of the Haihe River at 8 and 1 km, respectively from the Bohai Sea. Tributary sampling included animal feces storage lagoons at two swine farms (S3, S4), and two fishponds (S5, S6). Background antibiotic concentrations upstream of the putative pollution sources were very low, if detected, accounting for only $0.01-2\%$ of the concentrations measured downstream of the sources (i.e., $6-23\ ng/L$).

Surface water and surficial sediment samples were collected in triplicate during two synoptic sampling events, in August 2009 (high-flow period, sites M1–M9) and December 2009 (low-flow period, sites M1–M9 and S1–S6). Samples were collected into

sterile, aluminum-covered containers (to prevent photodegradation of antibiotics) and stored at 4 °C during transport to the laboratory, where water samples were stored at 4 °C and sediment samples were stored at −18 °C until sample pretreatment, which occurred within 24 h of the sample collection.

Sample Pretreatment and Solid-Phase Extraction. Sample pretreatment and solid-phase extraction of both water and sediment samples was carried out as described previously[18] (see Supporting Information for details). Briefly, in addition to using standard additions for quantification (see Analytical Method Validation section below), [trimethyl-^{13}C$_3$]caffeine (Cambridge Isotope Laboratories) was added to water and sediment samples as surrogate to compensate for matrix effects during both water and sediment pretreatment. Simatone (Sigma−Aldrich, Germany) was also used as internal standard to enhance analytical precision. Although use of multiple internal standards and/or surrogates is preferred to enhance the accuracy of analysis of multiple compounds with different physicochemical properties, unavailability of some labeled antibiotics and budget constraints precluded this approach. Because of such constraints, several recent studies similarly relied on the standards addition method plus a single internal standard to analyze multiple antibiotics.[33−37]

During pretreatment, water samples (500 mL) were spiked with 1 mL of 0.5 mg/L [trimethyl-^{13}C$_3$]caffeine and filtered through 0.45 μm glass fiber filters, and the pH was adjusted to 5 with citrate buffer (Tianjin University, China). Divalent cations (Ca^{2+}, Mg^{2+}, Cu^{2+}, and Mn^{2+}), which have affinity for tetracyclines and macrolides, were complexed by the addition of 0.2 g of disodium ethylenediaminetetraacetate (Na$_2$EDTA) to minimize interference during LC-MS/MS analysis. Simatone (100 μg/L) was also added prior to analysis. To avoid interference from dissolved organic matter, samples were also pretreated with Strata strong anion exchanger (SAX) cartridges followed by extraction with Oasis hydrophilic−liphophilic balance (HLB), as detailed in the Supporting Information.

[trimethyl-^{13}C$_3$]Caffeine (1 mL of 0.5 mg/L) was also added to 5 g of lyophilized and ground sediment samples, which were extracted with 30 mL of extraction buffer including 15 mL of methanol (Sigma−Aldrich, St. Louis, MO), 5 mL of 0.1 M Na$_2$-EDTA, and 10 mL of citrate buffer (pH 5). Simatone (100 μg/L) was then added to the extract. The mixture was vortexed for 1 min at 2500 rpm and extracted by ultrasonication for 15 min. The solution was centrifuged for 5 min at 4000 rpm, and 25 mL of supernatant was collected into a flask. The procedure was repeated three times by extracting three different aliquots of the initial sample and then combining the three supernatants for a volume of approximately 75 mL. The final supernatant mixture was blended and diluted to 500 mL with pure water.

High-Performance Liquid Chromatography−Tandem Mass Spectrometry Analysis. HPLC−MS/MS analysis was performed as described previously[18] (see Supporting Information for details, including Table S3). Briefly, the Intersil ODS-3 column (2.1 × 250 mm, 5 μm particle size; GL Sciences, Japan) was maintained at 40 °C, with injection volumes of 10 μL. Acetonitrile (phase A) and purified water with 0.3% formic acid (v/v) (phase B) were used as mobile phases at a total flow rate of 0.2 mL/min, with the following gradient: the contribution from A was linearly increased from 10% to 30% over 2 min and held constant for 4 min. Then A was increased to 35% over 4 min and maintained there for 5 min. The cycle was completed by returning A to the initial percentage (10%) over 4 min and maintaining for 6 min until the next injection.

Table 1. Average Antibiotic Concentrations in the Water Column Mainstream of the Haihe River

antibiotic[a]	avg concn (ng/L)	
	August 2009	December 2009
TMP	$(1.0 \pm 0.7) \times 10^2$	$(1.4 \pm 0.8) \times 10^2$
SD	$(1.4 \pm 0.8) \times 10^2$	$(1.7 \pm 0.8) \times 10^2$
SMZ	$(1.5 \pm 0.9) \times 10^2$	$(1.9 \pm 1.0) \times 10^2$
SCP	$(1.6 \pm 1.2) \times 10^2$	$(2.1 \pm 1.7) \times 10^2$
CIP	$(1.1 \pm 0.8) \times 10^2$	$(1.3 \pm 0.5) \times 10^2$
VFX	$(1.7 \pm 0.5) \times 10^2$	$(1.8 \pm 1.2) \times 10^2$
TC	$(2.6 \pm 0.3) \times 10^1$	$(2.7 \pm 0.3) \times 10^1$
OTC	$(4.1 \pm 0.2) \times 10^1$	$(4.0 \pm 0.0) \times 10^1$
ERY	$(3.4 \pm 1.1) \times 10^1$	$(3.8 \pm 0.6) \times 10^1$
ROX	$(2.7 \pm 1.4) \times 10^1$	$(3.7 \pm 0.8) \times 10^1$

[a] TMP = trimethoprim, SD = sulfadiazine,SMZ = sulfamethoxazole, SCP = sulfachloropyridazine, CIP = ciprofloxacin, VFX = ofloxacin, TC = tetracycline, OTC = oxytetracycline, ERY = erythromycin, and ROX = roxithromycin.

Analytical Method Validation. Recoveries of the 12 target compounds were determined for surface water and sediment by the standards addition method. Samples were spiked with different antibiotic concentrations (10, 50, 100, and 500 ng/L for surface water; 1, 10, 50, and 500 ng/g for sediment). Each sample was spiked with all 12 antibiotics, dissolved in a methanol stock solution, and analyzed in triplicate after extraction with 15 mL of methanol (Sigma−Aldrich, St. Louis, MO), 5 mL of 0.1 M Na$_2$EDTA, and 10 mL of citrate buffer (pH 5). The respective recoveries of sulfonamides, quinolones, tetracyclines, and macrolides were 71−92%, 60−82%, 65−87%, and 59−80% in water samples and 72−91%, 51−74%, 58−69%, and 67−69% in sediment samples. Recoveries of [trimethyl-^{13}C$_3$]caffeine for both water and sediment samples were 71−90% (Tables S4-1 and S4-2, Supporting Information).

Limits of detection were determined as the lowest concentration resulting in a signal-to-noise ratio (S/N) ≥ 3. Analytical method validation for water and sediment samples is described in the Supporting Information (Tables S4-1 and S4-2). Reproducibility of response [peak area relative standard deviation (RSD, %)] was calculated from triplicate injections for each of three samples spiked at the same concentration. RSD values ranged from 0.5% to 2.5% for water samples and from 0.5% to 5.0% for sediment samples.

Hydrological and Chemical Characteristics. The river flow rate and flow velocity were measured with a MGG/KL-DC flow meter (Kai Feng Kai Liu Instrument Co., Ltd.). Dissolved organic carbon (DOC) in the water column and total organic carbon (TOC) in the sediments were measured with a Shimadzu TOC5000 analyzer. Cation-exchange capacity (CEC) was measured by previously described methods.[38]

Antibiotics Attenuation Model. Antibiotics attenuation (e.g., by adsorption, dilution, photolysis, hydrolysis, and biodegradation) was assumed (and confirmed) to follow first-order kinetics. The attenuation coefficient (K) was calculated as[39]

$$K = \left(\frac{v}{L}\right) \ln \left(\frac{c_i}{c_I}\right) \qquad (1)$$

where L is the distance from site i to site I; c_i and c_I are the antibiotic concentrations at sites i and I, respectively; and v is the average river velocity between the two sites. K was determined without the use of conservative tracers that would be needed to quantify dilution. Thus,
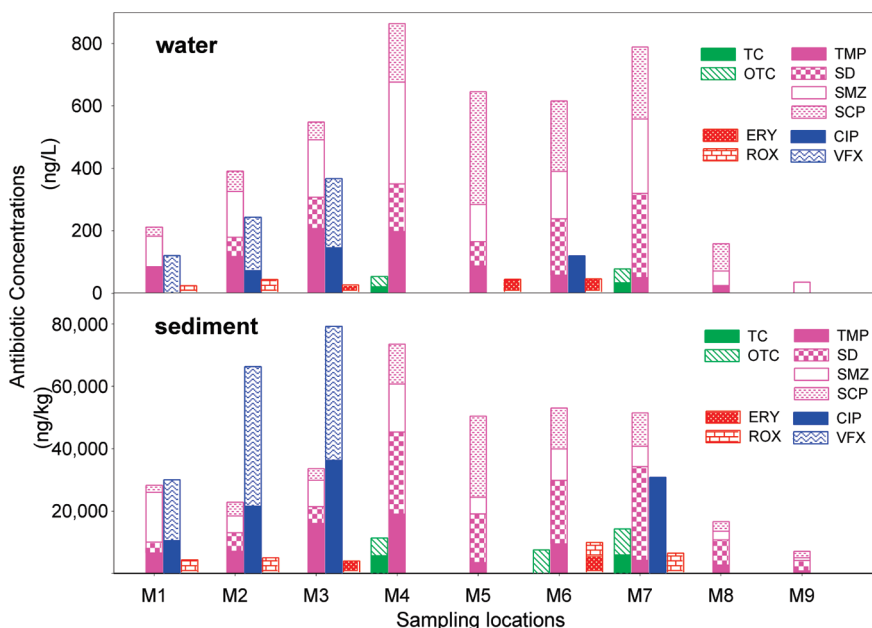
Figure 2. Spatial distribution of 12 antibiotics in the Haihe River main stream (August 2009). Colors reflect the four different classes of antibiotics: sulfonamides (pink), tetracyclines (green), macrolides (red), and quinolones (blue).
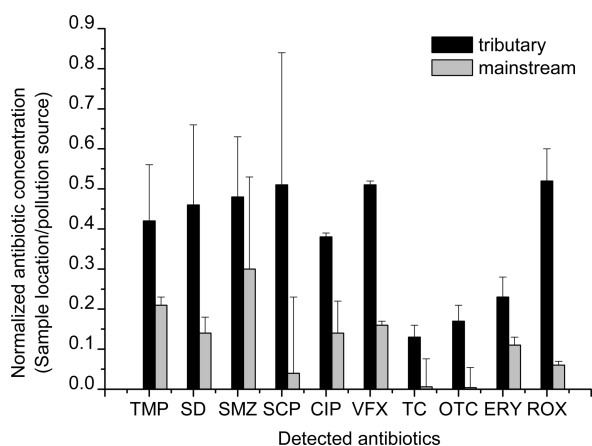

Figure 3. Relative antibiotic concentrations (normalized to corresponding pollution source concentrations) in Haihe River tributaries and the main stream in December 2009. Significantly higher concentrations were observed at the pollution sources, followed by concentrations in tributaries and in the main stream ($p < 0.05$). Error bars represent ± 1 standard deviation.
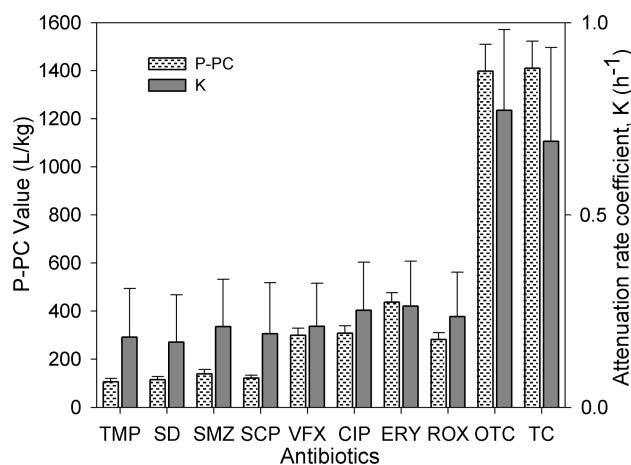

Figure 4. Attenuation coefficients and pseudopartitioning coefficients (P-PC) for antibiotics detected in the Haihe River basin. Results represent average values (± 1 standard deviation) for six tributary segments that were sampled in December 2009 (Table S7, Supporting Information).

K values represent overall attenuation coefficients that provide insight on the relative persistence and potential reach of different antibiotics (rather than specific degradation coefficients for use in predictive fate and transport models).

The first-order attenuation assumption was tested by assessing the goodness of fit of the data to the exponential decay model [$C = C_0 e^{-KL/v}$] as described in the Supporting Information (Table S7). This model was only applied to segments with a single pollution source, usually located in the tributaries.

Statistical Analysis. Antibiotic transport and attenuation in aqueous systems can be influenced by their physicochemical properties as well as hydrological (flow rate) and chemical factors such as pH, dissolved organic matter (DOM) in the water column, total organic matter (TOM) in sediments, and cation-exchange capacity (CEC) in sediments. These factors, which affect K, were measured (Table S7, Supporting Information) and

analyzed by multiple regression analysis using SPSS software. The standardized coefficient (β), which reflects the number of standard deviations the dependent variable increases or decreases with one standard deviation increase in the independent variable, was used to evaluate the influence of these factors on K for each of the 12 antibiotics at each of the six tributaries. A higher β value indicates more significant influence of a given factor on the K value. Negative β values indicate hydrological and chemical factors that hinder antibiotic attenuation. Significance was assessed at the 95% confidence level ($p < 0.05$).

■ RESULTS AND DISCUSSION

Antibiotics Occurrence in the Haihe River. Ten of the 12 antibiotics targeted were detected in surface water samples of the main Haihe River during both sampling events [sulfamethazine

Table 2. Multiple Regression Analysis (SPSS) of Hydrological and Chemical Factors Influencing Antibiotic Attenuation Coefficients (K)[a]

antibiotic[b]	N[c]	flow rate (11.0−53.0 m³/s)		water pH (6.2−7.6)		water DOC (2.5−4.8 mg/L)		sediment TOC (15.5−38.1 mg/g)		sediment CEC (165−338 mmol/kg)	
		β	p[d]	β	p[d]	β	p[d]	β	p[d]	β	p[d]
Sulfonamides											
TMP	12	0.68	**<0.01**	0.14	0.10	−0.37	**0.03**	−0.04	0.62	−0.06	0.53
SMZ	18	0.56	**0.01**	0.05	0.63	−0.47	**0.02**	−0.08	0.60	0.00	0.96
SD	12	0.59	**0.03**	0.24	0.08	−0.41	**0.04**	−0.05	0.70	−0.41	0.78
SCP	18	0.54	**<0.01**	0.06	0.50	−0.45	**0.01**	0.02	0.80	0.00	0.90
Quinolones											
CIP	12	0.70	**<0.01**	0.07	0.25	−0.35	**0.01**	−0.10	0.27	0.00	0.96
VFX	12	0.48	**0.05**	0.19	0.18	−0.45	0.07	−0.11	0.58	−0.17	0.36
Macrolides											
ERY	12	0.58	**0.05**	0.16	0.29	−0.42	0.12	−0.03	0.89	−0.11	0.59
ROX	18	0.57	**0.03**	−0.02	0.89	−0.42	0.07	−0.14	0.45	−0.01	0.97
Tetracyclines											
OTC	18	0.05	0.74	0.24	**0.04**	−0.10	0.50	0.54	**<0.01**	0.42	**0.01**
TC	12	0.20	0.40	0.19	0.20	0.26	0.30	0.51	**0.05**	0.48	**0.04**

[a] Measured ranges of hydrological and chemical factors are shown in parentheses. [b] TMP = trimethoprim, SMZ = sulfamethoxazole, SD = sulfadiazine, SCP = sulfachloropyridazine, CIP = ciprofloxacin, VFX = ofloxacin, ERY = erythromycin, ROX = roxithromycin, OTC = oxytetracycline, and TC = tetracycline. [c] N represent number of samples analyzed. [d] Significance of the effect of hydrological and chemical factors on K was assessed at the 95% confidence level ($p < 0.05$) and is indicated by boldface type.

(SM2) and enrofloxacin (ENR) were not detected] (Table S6, Supporting Information). Sulfonamides were the predominant antibiotics, detected with the highest frequencies (83−94%) and at the highest concentrations (210−385 ng/L). Relatively high concentrations of SCP (176 ± 118 ng/L with a peak concentration of 385 ng/L) were detected in M4−M7, where many swine farms, dairies, and fishponds are located. SCP is used only as a veterinary antibiotic in the Haihe area, suggesting its potential usefulness as a chemical marker for livestock source pollution in the Haihe River basin, an application similarly suggested for sulfamethazine in the Mekong Delta, Vietnam.[40] However, caution should be exercised about such inferences because SCP may also be discharged from veterinary industries and was detected (though at significantly lower concentrations) at urban sites M1−M3 and in wastewater treatment plant effluents (Table S6, Supporting Information).

Temporal and Spatial Distribution of Antibiotics in the Haihe River. Antibiotic concentrations in surface water were significantly higher ($p < 0.01$) during the winter season, except for TC and OTC (Table 1). Lower detected antibiotic concentrations in the summer can be attributed to higher runoff (increased flow rate), faster photolysis,[23,41] thermal degradation (summer water temperature was approximately 26 °C versus 5 °C in winter), and biodegradation associated with higher microbial activity during the summer season.[42−44]

Eight of the 12 targeted pharmaceutical compounds were detected at sites M1−M3 (urban areas with an approximate population of 3.8 million). CIP and ofloxacin (VFX), which are used in human applications, were detected with highest frequencies (67% to 100%) at urban locations M1−M3. In contrast, the veterinary antibiotics sulfamethoxazole (SMZ), SCP, and SD were detected with the highest frequencies (100%) at rural sites M4−M7, which are located in the vicinity of numerous animal

husbandry and aquaculture industry operations, including approximately 177 CAFOs and 66.7 km² of aquaculture farms that are often adjacent to the banks of the Haihe River. Significant concentrations of antibiotics were detected in river sediments compared to the directly overlying water column samples (Figure 2), indicating the importance of sorption as an attenuation mechanism and underscoring the significance of sediments as antibiotic reservoirs in aquatic environments.

Potential Sources of Antibiotics in the Haihe River. Antibiotic concentrations were analyzed in WWTP effluents, swine farm lagoons, and fishponds that discharge to tributaries of the Haihe River. Eight veterinary pharmaceuticals belonging to the tetracycline, sulfonamide, quinolone, and macrolide classes were detected in swine farms and fishponds at 0.12−47 μg/L (Table S6, Supporting Information). These concentrations are 1−2 orders of magnitude higher than those found in WWTP effluents, indicating significant contribution of veterinary pharmaceuticals to total antibiotic residues in this region. The observed decrease in antibiotic concentrations (normalized to average pollution source concentrations) along the flow direction from the putative source to the tributaries to the main stream (Figure 3) support the hypothesis that the antibiotics detected in the main stream originated from potential agricultural sources along the tributaries. The average concentration of each antibiotic at these sources was 1−2 orders of magnitude higher than that in the main river.

Antibiotic Attenuation. First-order attenuation rate coefficients (K values) for individual antibiotics were determined separately for each of six tributaries (Table S7, Supporting Information). The individual K values, averaged for the sampled segments (Figure 4), indicate that tetracyclines (K ranged from 0.39 to 1.22 h⁻¹) were most prone to attenuation, followed by macrolides (K ranged from 0.09 to 0.48 h⁻¹), quinolones (K ranged from 0.08 to 0.45 h⁻¹), and sulfonamides (K ranged from 0.01 to 0.44 h⁻¹).

Sorption to sediments is an important mechanism contributing to the relatively high attenuation capacity for tetracyclines and macrolides, as suggested by previous lab studies.[28,4546] Although antibiotic partitioning between water and river sediments is a dynamic process, the relative importance of sorption as an attenuation mechanism can be assessed by normalizing antibiotic concentrations in the water column by the corresponding concentrations in directly underlying sediments, to estimate pseudopartitioning coefficients (P-PC, liters per kilogram) (Figure 4). P-PC values were highest for tetracyclines (1410 L/kg for TC, 1398 L/kg for OTC), which likely contributed to the relatively low aqueous tetracycline concentrations (Figure 2). The significance of sorption for the relatively fast attenuation of tetracyclines (Figure 4) is also inferred by the high β values associated with sediment TOC and CEC (Table 2), which respectively facilitate sorption through cation bridging and cation exchange.[26−28]

The river flow rate exerted the most significant effect on the overall K values for sulfonamides, quinolones, and macrolides (Table 2), indicating that dilution plays an important role in the attenuation of these three antibiotic classes. Slower attenuation of macrolides, quinolones, and sulfonamides was significantly correlated with higher DOC (negative β values). Apparently, some functional groups in dissolved organic matter (e.g., carboxyl, hydroxyl, carbonyl) facilitate electrostatic association with these antibiotics and enhance their migration by cotransport, as previously reported.[47]

Overall, this work shows a predominant presence of sulfonamides in the Haihe River basin, which are discharged to its tributaries from swine farm lagoons and fishponds. This underscores the need to consider more efficient practices for management of livestock waste and aquaculture drainage and their treatment prior to reuse or discharge. Given the persistence and high migration propensity of sulfonamides, further research is also needed to better understand their fate in the environment, their impact on the establishment and amplification of antibiotic resistance reservoirs, and the associated risks to public health.

ASSOCIATED CONTENT

S **Supporting Information.** Additional text and seven tables describing structure and physicochemical properties of the 12 antibiotics investigated in this study, information on wastewater treatment plants in Tianjin, details on sample extraction and pretreatment, antibiotic concentration data at different sampling locations and times, analytical methods validation, determination of first-order attenuation rate coefficients, and data for hydrological and chemical variables that may influence antibiotic distribution. This material is available free of charge via the Internet at http://pubs.acs.org.

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