The distribution of antibiotics in water of a river basin in South China

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Abstract. In water environment field, one of the most attractive research topics is the determination of contamination characteristics of antibiotics in water. In order to investigate the distribution of antibiotics in surface water and drinking water of a certain river basin in southern China, we determined the types and concentrations of antibiotics that contaminated the river by performing HPLC-ESI- MS/MS method. Thus, we detected 17 antibiotics in four surface water samples (B1, B2, B3, and B4). In sampling points B3 and B4, we detected 16 antibiotics separately. The detection rates of norfloxacin, ofloxacin, and erythromycin-H_2O were 100%, and the antibiotic erythromycin-H_2O had the maximum concentration. In six drinking water samples (A1, A2, A3, A4, A5, and A6), we detected 13 antibiotics. In A5 water samples, we detected all the 13 antibiotics. The detection rate of ofloxacin and erythromycin-H_2O was 100%, and erythromycin-H_2O was the antibiotic with the highest concentration. We also found that from the upstream to the downstream of the river basin, the types of antibiotics in river increased gradually. In the upstream water samples (B1), we detected three antibiotics. Erythromycin-H_2O was the antibiotic with the highest concentration of 6.61 ng/L, and sulfapyridine had the lowest concentration of 2.82 ng/L. In the downstream water samples (B4), we detected 16 antibiotics. Erythromycin-H_2O was the antibiotic with the highest concentration of 277.58 ng/L, and the Sulfamonomethoxine was the antibiotic with the second-highest concentration of 242.1 ng/L. In addition, different membrane treatment processes could remove different amounts of antibiotics from the water samples. The study is an important reference for providing environmental protection to river water basin.

1. Introduction

An antibiotic is a class of drugs that can either inhibit the growth of bacteria or kill the bacteria. In clinical practice, antibiotics have been extensively used to treat both humans and livestock animals as they have prophylactic, therapeutic, and growth-promoting properties [1, 2]. China is an important country of interest in this study given the massive production and use of antibiotics in the country. In 2013, the total consumption of antibiotics was estimated to be 162 thousand tons in China, wherein 48% of total antibiotics were consumed by humans, while the remaining antibiotics were administered to veterinary patients [3]. In generic terms, the consumed antibiotics are continuously discharged into the natural ecosystems *via* excretion (urine and feces) following their short time of residence in the bodies of humans and animals. Then, these drugs are included in the aquatic environment when the effluents from the wastewater treatment plant are discharged into either surface water or into

groundwater. The various avenues for the discharge of effluents are as follows: the leachate in landfill, leaching and runoff from farmlands fertilized with manure, sewage disposal in agricultural areas, and other industrial discharges [4, 5]. In China, environmental scientists have detected antibiotics in all kinds of aquatic environments across the country. Antibiotics in aquatic environments may pose as a potential ecological risk to aquatic ecosystems. Therefore, most current research studies focused on investigating the river basin water environment also explore the impact and concentration of antibiotics in the aquatic environment [6].

The use of antibiotics is greater in southern China than in the North because the economy is more developed and the population density is also greater in southern China [7]. Zhang Q *et al.* found that antibiotics were detected in the Huangpu River, the Yangtze River Estuary etc., with the detectable amount of sulfonamides being the greatest [8]. Ye J P *et al* found that antibiotics were also detected in the Pearl River Delta and other places, and the concentration of these antibiotics in these water bodies was in the range 10–100 ng/L [9]. Therefore, we performed a high performance liquid chromatography – tandem mass spectrometry (HPLC-MS/MS) to detect antibiotics in the water (surface water and drinking water) of a river basin in South China. Thus, we studied the kinds and the distribution of antibiotics in the river basin. The results of the analysis served as reference standards for implementing policies pertaining to water environment protection.

2. Materials and methods

2.1. Sampling
In total, we identified 10 sampling points from the region extending from the upper reaches of the river basin to the lower reaches of the river, including four rivers and six residents' drinking water source. Thus, we collected 5 L water samples from each sampling point. The river water was the surface water, while the drinking water was the residents' tap water at home. After sampling, we added 2 ml methanol and the pH was adjusted to 3 by adding 3 mol/L H_2SO_4. Then, we placed the sample in the brown bottle. The sample was carried back to the lab that day and stored at 4°C before performing detection. Figure 1 illustrates a layout of sampling points in the watershed.
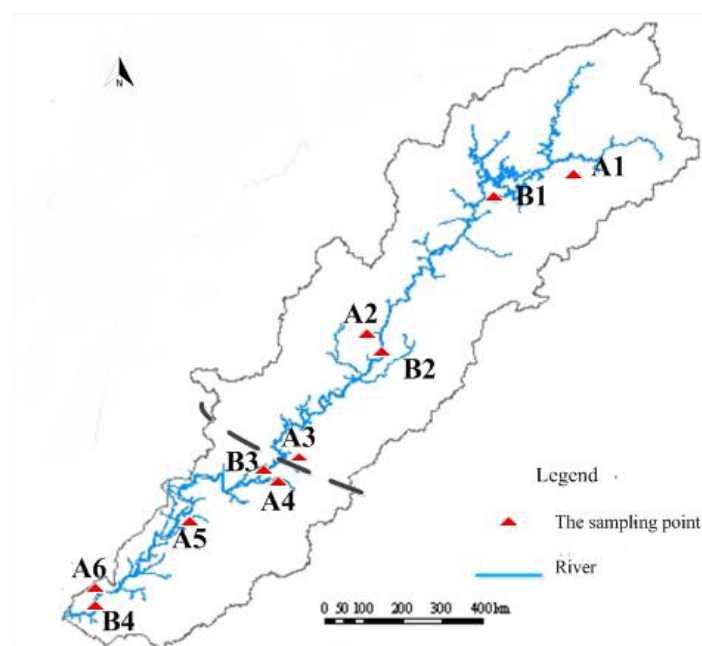

Figure 1. Map of the sampling sites in the river.

2.2. Sample preparation and analysis

The water samples (Sample volumes were 2 L.) were filtered through 0.45 µm filter membranes. We added 0.4 g disodium ethylenediamine tetraacetate (Na_2EDTA) and 200 ng of $^{13}C_3$-caffeine into filtered water; this combination of chemicals served as a surrogate monitoring the recovery of the sample from the extract. Then, the filtered water samples were extracted by performing solid-phase extraction (SPE) using Oasis HLB cartridge (500 mg; 6 mL) were provided by waters (Milford, MA, USA). The cartridge was eluted with 6 mL methanol. After extraction, we dried the HLB column under nitrogen gas at room temperature. The analytes were collected in a 10 mL brown glass vial, and then we dissolved it in 40% aqueous methanol to produce a final volume of 1.0 mL [10, 11].

An ultra-performance liquid chromatography (UPLC)–tandem mass spectrometry detection system was operated in the positive ion mode of electrospray ionization (ESI) source to detect and analyze pretreatment water samples. To perform qualitative and quantitative analysis of antibiotics, we used the multiple reflection detection method.

3. Results and discussion

3.1. Analysis of types of antibiotics in water
As shown in table 1, seventeen antibiotics were detected in total and they were classified into six types, were detected. Among them, the detection rate of ofloxacin and dehydrated erythromycin was 100%.

Table 1. Concentrations of antibiotics detected in water samples collected from drinking water and river water (ng/L).

| Category	Compound	Drinking water						River water			
		A1	A2	A3	A4	A5	A6	B1	B2	B3	B4
Fluoroquinolones	Ciprofloxacin	6.27	-	4.63	-	5.02	-	-	5.49	4.82	-
	Norfloxacin	3.47	-	4.79	-	4.4	-	2.82	6.74	3.06	5.4
	Ofloxacin	12.64	4.81	11.79	7.26	8.7	11.27	6.61	15.67	6.39	9.43
Macrolides	Dehydrated erythromycin	1.84	1.39	15.73	1.91	82.52	2.62	6.47	11.6	66.48	122.7
	Kitasamycine	-	-	-	-	2.57	-	-	-	1.46	2.84
	Tylosin	-	-	-	-	1.89	-	-	-	0.26	13.08
Tetracyclines	Doxycycline	-	-	-	-	-	-	-	-	4.83	4.25
	Oxytetracycline	-	-	-	-	17.38	-	-	-	72.59	138.8
Lincosamides	Lincomycin	-	-	-	-	-	-	-	-	15.63	128.6
Polyethers	Salinomycin	-	-	-	-	9.42	-	-	-	11.26	4.46
Sulfonamides	Sulfadiazine	-	-	-	-	-	-	-	-	4.38	5.15
	Sulfamethazine	-	-	-	-	32.78	-	-	-	39.91	37.78
	Sulfamethoxazole	-	-	-	-	22.47	-	-	1.03	13.15	94.01
	Sulfamonomethoxine	-	-	-	-	60.11	-	-	5.63	27.01	242.1
	Sulfapyridine	-	-	-	-	1.65	-	-	-	1.91	2.41
	Suifoquinoxaline	-	-	-	-	-	-	-	-		12.56
	Trimethoprim	-	-	-	-	8.77	-	-	-	2.28	22.32

-: Not detected.

The results were as follows: 1) antibiotics were detected in all the sampling sites, and the types of antibiotics detected in river water were significantly more than that in drinking water; 2) in drinking water sampling points, we detected tetracyclines, polyethers, and sulfonamides in A5, but the same compounds could not be detected in other drinking water sampling points. Moreover, highest amount of dehydrated erythromycin of macrolides was detected only in A5 and not in other drinking water sampling points; 3) in river water sampling points, minimum species of antibiotics were detected in B1, and fluoroquinolones and macrolides were the antibiotics that were mainly detected in the river water

sampling points.

Different kinds of water purification processes are used presently, and the extent of removal of antibiotics in water bodies depends on the method selected for water purification. Due to this reason, the types and contents of antibiotics in drinking water are significantly lower than those in rivers. In different regions of the world, the amount and variety of antibiotics in drinking water will be affected by water purification process and water quality.

3.2. Distribution of antibiotics in the river

After sampling at the upper stream, midstream, and downstream sections of the river, we determined the contents of antibiotics in every sampling site. Figure 2 illustrates the distribution of antibiotics in the river basin. As shown in figure 2, antibiotics existed in the entire reach of the river; fluoroquinolones and macrolides were the antibiotics that were mainly detected in the river. In the entire river, a 100% detection rate was observed for the following antibiotics: dehydrated erythromycin, ofloxacin, and norfloxacin. The concentrations of erythromycin, ofloxacin, and norfloxacin were in the range of 6.47–122.7 ng/L, 2.82–6.74 ng/L, and 6.39–18.85 ng/L, respectively.
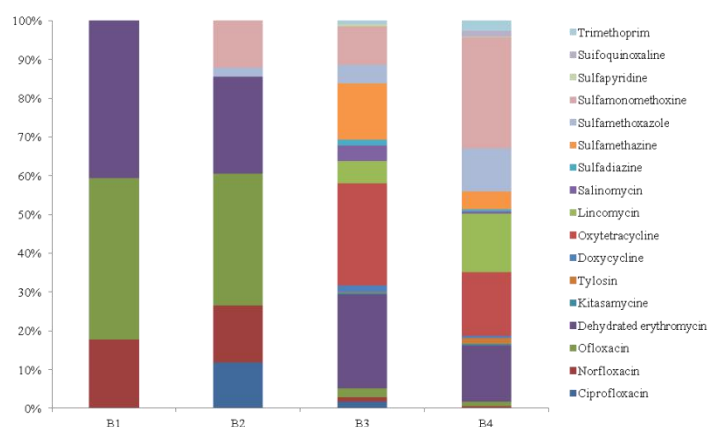
Figure 2. The proportion of antibiotics in the river sampling points.

In the upstream of river water, we detected three kinds of antibiotics. Out of the total amount of antibiotics detected in the river water, the proportion of norfloxacin was 17.7%. The concentration of norfloxacin was 2.82 ng/L. Dehydrated erythromycin accounted for 40.7% of the total amount of antibiotics detected in river water. The concentration of dehydration erythromycin was 6.47 ng/L. The proportion of ofloxacin was 41.6% in the total amount of antibiotics detected in river water; the concentration of ofloxacin was 6.61 ng/L. We detected 16 kinds of antibiotics in the middle and lower reaches of the river. The detection rate of sulfonamides was the highest, accounting for 49.21% of the total amount of antibiotics detected in the sample. The concentration of sulfamonomethoxine was 242.1 ng/L in the sample, accounting for 58.2% of the total sulfonamides. In the downstream of river water, ciprofloxacin was not detected. Sulfapyridine was the antibiotic that was present in least amounts in the sample; the concentrated of the detected sulfapyridine was 2.41 ng/L.

Based on the detection results, we conclude there is a certain relationship between the concentration of antibiotics and human activities. In the upper reaches of the watershed, villages were more dispersed and the impact of human activities on the environment was small; therefore, the types and contents of antibiotics being detected were less. In the middle and lower reaches, the impact of human activities on the environment was relatively large because of the dense population, developed industry and agriculture, and the vast distribution of many fisheries in this region. As a result, the types and contents of antibiotics being detected in this region were also more.

Along the river, there was steady increase in the kinds of antibiotics detected in water. The contents of the antibiotics being detected also increased steadily along the river, and this increase in the content

and types of antibiotics is closely related to human activities; however, the influence of river water quality, hydrology, and other factors is also significant in this case. Meanwhile, the lower reaches of water also contained the afflux of a tributary. If antibiotics existed in the tributary, then it would cause a certain impact on the downstream water.

3.3. Distribution of antibiotics in drinking water

We collected drinking water samples in the rural areas along the river, and the distribution of antibiotics in the drinking water is shown in figure 3. Antibiotics were detected in the drinking water collected from different areas, and fluoroquinolones and macrolides were the two antibiotics that were primarily detected in various drinking water samples. The detection rate of dehydrated erythromycin and ofloxacin was 100% in drinking water, and the concentration of dehydrated erythromycin and ofloxacin was in the range of 1.39–82.52 ng/L, 4.81–12.64 ng/L, respectively. During the analysis of drinking water samples, we detected 2-4 kinds of antibiotics in the rest sampling sites. In addition, we also detected 13 kinds of antibiotics in A5 sampling sites.
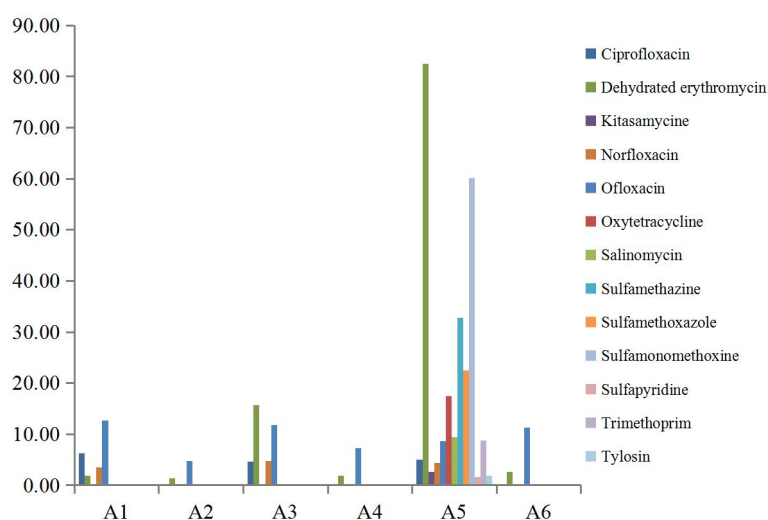
Figure 3. The distribution of antibiotics in the drinking water.

Through a survey, we found that drinking water sources of A2-A5 were all river water, and all the sources except A5 were subjected to conventional processes. An integrated water purification process was followed for the source A5. The effects were not the same as different plant processes were adopted for antibiotics treatment. Therefore, as the A5 water sample had the highest amount of antibiotics, it was more likely to be affected by the water treatment process. In addition, the water quality of water supply would have an impact on drinking water. Because A5 water plant was located in the lower reaches of others, the types of antibiotics in this water sample were significantly more than that detected in the upper reaches of the river.

The contents and types of antibiotics in drinking water were closely associated with the water quality of drinking water sources and water treatment process. Furthermore, the pollution from the municipal water supply network also affected the quality of drinking water.

4. Conclusions

- We detected 13 kinds of antibiotics in drinking water. The distribution of ofloxacin and dehydrated erythromycin was wide in drinking water, and the detection rate of these antibiotics was 100%. Moreover, water purification process certainly governed the extent of removal of antibiotics in raw water. Thus, the treatment effect of different wastewater treatment plants was not the same, the removal efficiency of conventional process was better.

- In the river water, we detected 17 kinds of antibiotics; the distribution of dehydrated erythromycin, ofloxacin, and norfloxacin was wide, and the detection rate of these antibiotics was 100%. From upstream to downstream, there was a steady increase in the content and species of antibiotics in water. This is because human activities also steadily increased as we moved from upstream to downstream of the river.

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