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RESEARCH ARTICLE

Prioritization, sources, and ecological risk of typical antibiotics in the Huai River, a Chinese major river: a warning about aquaculture

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Abstract

This is the first comprehensive report on antibiotics in the Huai River, a major Chinese river. To illuminate the concentrations, prioritization, spatial distributions, sources, and ecological risks of antibiotics, surface water samples were collected and three types of most widely used antibiotics (16 sulfonamides, 8 tetracyclines, and 14 quinolones) were analyzed. The results indicated that concentrations of ∑quinolones (86 ± 31 ng/L) > ∑tetracyclines (20 ± 13 ng/L) > ∑sulfonamides (11 ± 3.7 ng/L). Oxolinic acid (OXA), cinoxacin(CINX), norfloxacin (NFX), and methacycline (MTC) were the priority antibiotics with mean concentrations > or close to 10 ng/L, however, they were rarely included as target compounds in most previous Chinese investigations. Different spatial distributions of antibiotics were discovered across three reaches separated by two sluices, demonstrating that the sluices may impact antibiotic dissemination. According to the results of the source analysis, the aquaculture industry was the major source of observed antibiotics (49%), followed by livestock & poultry farming (26%) and mixed sources (25%). Because commercial fishing in the Huai River has been prohibited, the aquaculture industry will expand in the next years, and antibiotic contamination caused by the aquaculture industry deserves more attention. The risk quotients were calculated by comparing observed antibiotics to predicted no-effect concentrations, and the results showed that observed antibiotics posed negligible or low integrated risks for *Green algae*, and medium or low integrated risks for *Daphnia magna*.

Keywords Quinolones · Tetracyclines · Sulfonamides · Surface water · Huai river

Introduction

Antibiotics are emerging pollutants that have aroused increasing concerns due to their widespread dispersion in numerous environmental compartments (Huang et al. 2020; Li et al. 2020b). Once released into the environment, antibiotics will be able to promote the development of antibiotic-resistant bacteria as well as the dissemination of antibiotic resistance genes, posing major hazards to ecosystems and human health (Huang et al. 2020; Li et al. 2020b; O'Neill 2016; Qiao et al. 2018). By 2050, antibiotic resistance is expected to kill around 10 million people (Huang et al. 2020; Li et al. 2018a). China is the world's greatest antibiotic producer and consumer (Zhang et al. 2015). In 2013, China consumed around 162 000 tons of antibiotics, which is approximately 9 times more than the world's second-largest user, the United States (Huang et al. 2020; Zhang et al. 2015). China's daily antibiotic dosage per 1000 residents was approximately five times higher than that of European countries and the United States (Ying et al. 2017).

Antibiotics in the aquatic environment, even in trace amounts, might have direct and/or indirect harmful effects on aquatic and sediment-dwelling organisms (Gu et al. 2019; Hu et al. 2018; Lu et al. 2022; Zhou et al. 2021). Furthermore, the aquatic environment is regarded as a key environmental reservoir of antibiotic resistance genes, and it plays a crucial role in the development and spread of these genes (Hooban et al. 2020; Zhang et al. 2021). Several studies have been conducted over the last decade to investigate

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the presence of antibiotics in Chinese aquatic environments (Huang et al. 2020; Li et al. 2020b; Liu et al. 2018). According to two recent review studies, around 100 antibiotics have been identified in China's surface water (Huang et al. 2020; Li et al. 2020b). Most previous research, however, concentrated on rivers and lakes in eastern and southern China, such as the Pearl River, Yangtze River, Hai River, and Taihu Lake. Antibiotic distribution and ecological risks in the aquatic environment of central and western China are still unclear. Furthermore, only a few types of antibiotics were previously analyzed for each study (typically a dozen or more), and the pattern and priority of antibiotics still need to be investigated further.

The Huai River, one of China's seven major rivers, flows across East China, with the Yellow River to the north and the Yangtze River to the south. It has a catchment area of approximately 270,000 km^2 and serves around 190 million people. The Huai River Basin is a traditional agricultural area with about 120, 000 km^2 of farmland. Furthermore, livestock & poultry breeding and aquaculture are also well-developed. So far, just a few research on antibiotics in the Huai River have been conducted (Huang et al. 2020; Li et al. 2020b). Antibiotic concentrations, priority, geographical distributions, sources, and ecological risks in the river are not thoroughly characterized. Surface water samples were collected in the Middle Section of the Huai River (MSHR) to complete these information gaps, and three kinds of antibiotics (16 sulfonamides, 8 tetracyclines, and 14 quinolones) were analyzed in these samples. This is, to the best of our knowledge, the first thorough report on antibiotics in the Huai River.

Materials and methods

Sample collection

Geographically, the Huai River is separated into three sections: upper, middle, and lower, which are largely in Henan, Anhui, and Jiangsu Provinces, respectively. The middle section of the watershed accounts for around 80% of the overall watershed area and provides perfect sailing conditions;. Single samples were collected at each site ($n = 32$) during December 2 to 11 of 2018 (Fig. 1). Details of the sampling information can be found in the Supplementary Material (SM, Table S1). The average distribution approach was used

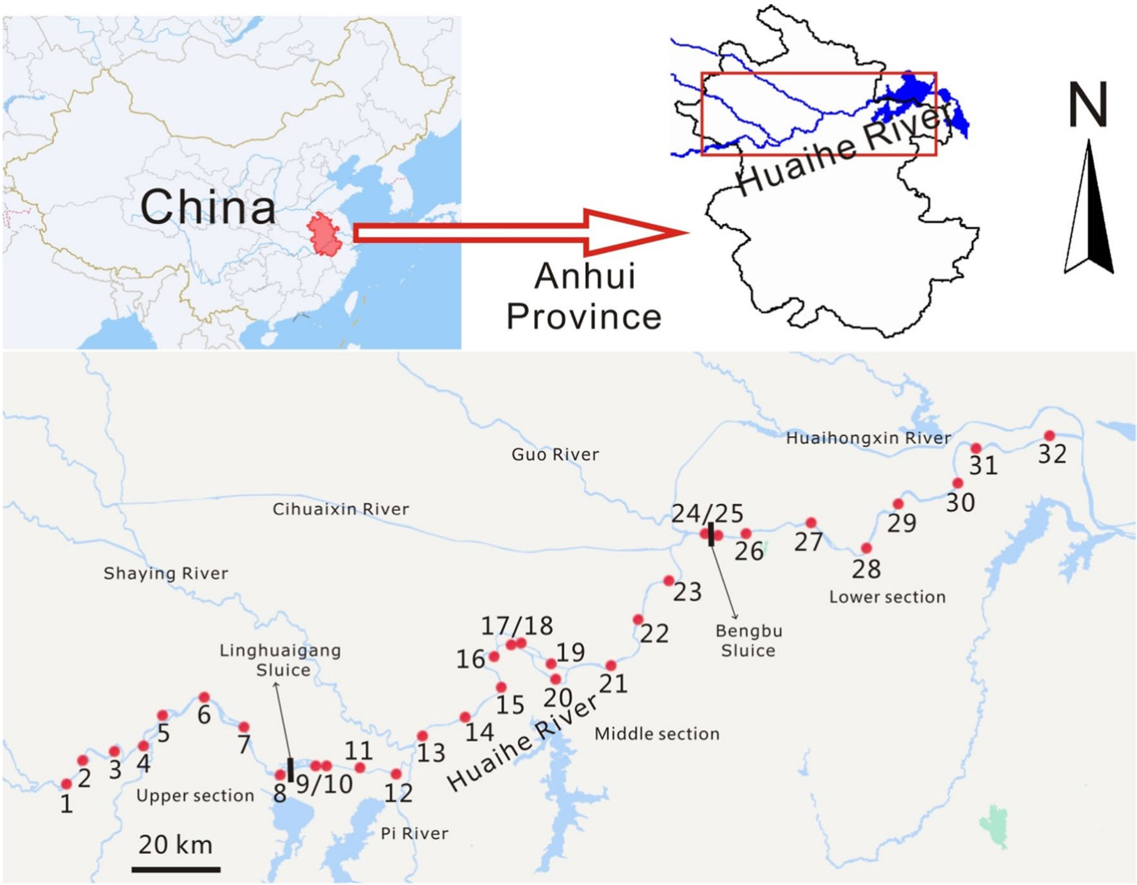

Fig. 1 Sampling Map

to lay out the sampling locations, and the spacing between each sampling site was around 5–8 km. Surface water samples were collected with amber glass bottles (pre-cleaned with acetone, methanol, and Milli-Q water and rinsed with water samples) in the center of the river by a sampling ship. The water samples were then treated with Na_2EDTA (0.5%, w/v), pH adjusted to 3.0 ± 0.5, and filtered using glass fiber filters (GF/F, Whatman, 0.7 μm) to remove particles. Thereafter, the samples were stored at 4 °C and pretreated within 2 days.

Sample pretreatment and analysis

All of the solvents used in this study were HPLC grade and bought from Merck & Co Inc, and Milli-Q water was utilized. Standard solutions of antibiotics were purchased from O_2si Smart Solutions, and C^{13} labeled caffeine was purchased from Cambridge Isotope Laboratories, Inc.

C^{13} labeled caffeine was added to the samples before extraction. Water samples (1L) were then extracted using Oasis HLB 6 cc (500 mg) solid-phase extraction (SPE) cartridges at a flow rate of 5 ml min^{-1}. 20 mL of methanol and 6 mL of Na_2EDTA solution (0.5% w/v, pH = 3) were used to precondition the HLB cartridges. After loading the water samples, the cartridges were washed with 10 mL of Milli-Q water, vacuum-dried, and eluted with 12 mL methanol. The eluent was concentrated to 1 mL under nitrogen and kept at -20 °C before analysis.

Antibiotics were analyzed using an LC/MSMS system (Shimadzu LC-20A liquid chromatography interfaced with an AB Sciex API 4000 + triple quadrupole mass spectrometer) with an electrospray ionization source (ESI +) using multiple reaction monitoring (MRM). An Agilent RRHD Zorbax Stable Bond-C18 (2.1 × 100 mm, 1.8 μm) chromatographic column with a guard column (Agilent UHPLC Guard Stable Bond-C18, 2.1 mm, 1.8 μm) was used in the analysis. Detailed instrument settings and parameters are presented in the SM (Text S1). A list of analyzed antibiotics, and their abbreviations and CAS numbers are presented in Table 1.

Quality assurance and quality control (QA&QC)

Procedural and sampling blanks, as well as matrix spike tests, were performed for QA&QC. Means of matrix spiking recovery rates ($n = 5$) were 71–100% for sulfonamides, 51%-91% for tetracyclines, and 62–79% for quinolones, and all the relative standard deviations were < 14%. The surrogate recovery of C^{13} labeled caffeine was 54–94% (mean = 73 ± 9.3%). R^2 values of the calibration curves were > 0.99 for all the analyzed targets. The method detection limits (MDLs) were determined by using 10 times the signal-to-noise ratios and the concentration ratios of 1000 (1L water sample to 1 mL). Finally, the MDLs were 0.0019–0.061 ng/L sample for sulfonamides, 0.070–2.3 ng/L sample for tetracyclines, and 0.0088–0.84 ng/L sample for quinolones (Table S2). All the antibiotics in the procedural ($n = 5$) & sampling blanks ($n = 3$) were less than the MDLs, showing that no contamination occurred during sample collection, transportation, storage, and preparation.

Ecological risk assessment

The risk quotient (RQ) method was used to reflect the potential ecological risk caused by antibiotics.

$$\mathrm{RQ} = \mathrm{MEC_i}/\mathrm{PNEC_i} \tag{1}$$

$$\mathrm{RQ_{mix}} = \sum_{i=1}^{n} \mathrm{RQ_i} \tag{2}$$

where MEC and PNEC were the measured environmental concentration and predicted no-effect concentration of an antibiotic ($_i$). The PNECs were extrapolated using the 50% lethal concentrations (LC50) or 50% effect concentration (EC50) and an assessment factor (Bjerregaard et al. 2022).

$$\mathrm{PNEC} = \mathrm{LC50orEC50}/\mathrm{assessmentfactor} \tag{3}$$

Generally, the environmental toxicity data can be obtained through experimental toxicity tests (Park and Choi 2008). But, this kind of data is scarce for antibiotics. The other option is to simulate and calculate the environmental toxicity data according to the molecular characteristics of chemicals, such as the Ecological Structure Activity Relationships (ECOSAR) Class Program, which is developed by USEPA (United States Environmental Protection Agency, 2022). However, there might be a great difference between the simulated values and experimental values, sometimes, this difference can be several orders of magnitude (Qi et al. 2022). In this study, if the experimental toxicity data were available, these data were used (acquired through the ECOTOXicology Knowledgebase), otherwise, computational toxicology data were used (acquired through the ECOSAR) (Olker et al. 2022; United States Environmental Protection Agency, 2022).

Correlation analysis and positive matrix factorization (PMF)

To investigate the relationship between different antibiotics, correlation analysis was performed using Microsoft Excel 2010 software. In this study, the PMF approach (PMF 5.0 software obtained from the USEPA's official website) was used to reduce data dimensions and analyze antibiotic sources.

Table 1 Descriptive analyses of antibiotics in water of the Huaihe River (ng/L)

Compounds	Abbreviation	CAS No	Range	Mean ± SD	Frequency
Sulfacetamide	SAA	144–80-9	ND[1]-1.1	0.14 ± 0.20	29/32
Sulfamethazine	SMZ	57–68-1	ND-1.9	0.48 ± 0.63	30/32
Sulfadiazine	SDZ	68–35-9	ND-0.59	0.22 ± 0.15	27/32
Sulfathiazole	STZ	72–14-0	ND-0.49	0.082 ± 0.090	27/32
Sulfapyridine	SPD	144–83-2	ND-0.82	0.20 ± 0.19	30/32
Sulfamerazine	SMR	127–79-7	ND-0.20	0.025 ± 0.036	18/32
Sulfamethoxypyridazine	SMP	80–35-3	0.51–3.2	1.5 ± 0.58	32/32
Sulfameter	SME	651–06-9	ND-0.10	0.025 ± 0.025	15/32
Sulfamethizole	SMT	144–82-1	ND-0.41	0.093 ± 0.081	28/32
Sulfachloropyridazine	SCP	80–32-0	0.46–8.9	1.7 ± 1.6	32/32
Sulfadoxine	SDX	2447–57-6	ND-0.056	0.026 ± 0.017	23/32
Sulfamethoxazole	SMX	723–46-6	2.4–12	5.3 ± 2.4	32/32
Sulfamonomethoxine	SMM	1220–83-3	0.11–0.68	0.37 ± 0.15	32/32
Sulfisoxazole	SFX	127–69-5	ND-0.26	0.071 ± 0.057	25/32
Sulfadimethoxine	SDM	122–11-2	ND-0.30	0.080 ± 0.064	26/32
Sulfaphenazole	SPA	526–08-9	ND-0.53	0.22 ± 0.15	27/32
∑sulfnomides			**4.8–21**	**11 ± 3.7**	
Minocycline	MNC	10118–90-8	ND-3.8	1.5 ± 0.77	31/32
Oxytetracycline	OTC	79–57-2	ND-2.2	0.88 ± 0.62	30/32
Tetracycline	TC	60–54-8	ND-0.56	0.10 ± 0.14	5/32
Demeclocycline	DMC	127–33-3	ND-18	5.0 ± 4.7	30/32
Methacycline	MTC	914–00-1	ND-50	9.8 ± 14	29/32
Doxycycline	DXC	564–25-0	ND-4.7	1.1 ± 1.1	23/32
4-epi-anhydrotetracycline	ETC	7518–17-4	ND-6.8	1.3 ± 1.0	1/32
Anhydrotetracycline	ATC	1665–56-1	ND-1.7	0.71 ± 0.24	2/32
∑tetracyclines			**7.1–55**	**20 ± 13**	
Nalidixic acid	NXA	389–08-2	ND-6.2	3.7 ± 1.2	31/32
Flumequine	FMQ	42835–25-6	3.4–13	7.7 ± 2.3	32/32
Oxolinic acid	OXA	14698–29-4	0.51–63	26 ± 18	32/32
Cinoxacin	CINX	28657–80-9	ND-39	19 ± 12	25/32
Pipemidic acid	PMA	51940–44-4	ND-16	3.5 ± 4.9	15/32
Norfloxacin	NFX	70458–96-7	ND-52	14 ± 13	29/32
Enoxacin	ENX	74011–58-8	1.3–13	4.8 ± 2.4	32/32
Ciprofloxacin	CFX	85721–33-1	ND-16	2.0 ± 3.0	24/32
Pefloxacin	PFX	70458–92-3	ND-17	3.2 ± 3.3	31/32
Lomefloxacin	LFX	98079–51-7	ND-0.55	0.12 ± 0.10	5/32
Danofloxacin	DFX	112398–08-0	ND-6.3	1.7 ± 1.7	26/32
Enrofloxacin	EFX	93106–60-6	ND-1.4	0.36 ± 0.33	28/32
Ofloxacin	OFX	82419–36-1	ND-5.7	0.94 ± 1.2	18/32
∑quinolones			**32–150**	**86 ± 31**	

[1] ND means the concentration was < MDL

Result and discussion

Antibiotics in surface water of Huai River

Except for sarafloxacin, all of the targeted antibiotics, including 16 sulfonamides, 8 tetracyclines (6 tetracyclines and 2 transformation products), and 13 quinolones, were detected in surface water samples (Table S3-S5). Sulfonamides, tetracyclines, and quinolones are the most commonly used antibiotics in China, accounting for 12%, 14%, and 15% of overall consumption, respectively (Hu et al. 2018). In this study, concentrations of ∑sulfonamides, ∑tetracyclines, and ∑quinolones in surface water were 4.8–21 ng/L (mean = 11 ± 3.7 ng/L), 7.1–55 ng/L (mean = 20 ± 13 ng/L), and 32–150 ng/L (mean = 86 ± 31 ng/L). So, the contamination of these three types of antibiotics ranked:

quinolones > tetracyclines > sulfonamides (Table 1). This finding was consistent with a previous nationwide investigation, which discovered that quinolones were the most prevalent antibiotics in most Chinese rivers (Zhang et al. 2015). Individual antibiotics with the highest mean concentrations were OXA (mean = 26 ± 18 ng/L), CINX (mean = 19 ± 12 ng/L), and NFX (mean = 14 ± 13 ng/L) (Table 1). Except for the antibiotics mentioned above, the mean of MTC (9.8 ng/L) was near 10 ng/L, while the means of the other antibiotics were 10 ng/L or even 5 ng/L. (Table 1).

Geographically, the research area may be divided into three sections based on the river's two sluices (Fig. 1). The majority of tributaries, including the Shaying River, Pi River, Cihuaixin River, and Guo River, enter the Huai River mostly in the middle section. The means of ∑quinolones in the middle (98 ± 25 ng/L) and lower sections (98 ± 31 ng/L) were comparable but about twice as high as that in the upper section (52 ± 12 ng/L, Fig. 2a). Furthermore, ∑quinolones accounted for around 80% of ∑antibiotics in the middle and lower sections, whereas ∑quinolones accounted for approximately 50% of ∑antibiotics in the upper section. Thus, there was an evident import of quinolones from the middle section. The spatial distribution of ∑tetracyclines, on the other hand, showed a distinct tendency, with the average concentration in the upper section (33 ± 7.6 ng/L) being higher than those in the middle and lower sections (18 ± 13, and 13 ± 4.3 ng/L, Fig. 2a). Similarly, ∑sulfonamides in the upper section (14 ± 3.6 ng/L) were higher than those in the middle and lower sections (10 ± 3.4, and 8.4 ± 2.0 ng/L, Fig. 2a). These results revealed that the sources of antibiotics were different in these sections and that the sluices would restrict or perhaps prohibit antibiotic dissemination along the river.

Quinolones

As stated previously, the mean concentrations of OXA, CINX, and NFX were the highest among all antibiotics detected (Table 1 and Fig. 2b). Geographically, high concentrations of OXA and NFX were mainly detected in the middle and lower sections, while high concentrations of CINX were detected in all sections of the river. The most commonly used quinolones in China were OFX, CFX, NFX, and EFX, with total usages exceeding 5000 t in 2013 (Zhang et al. 2015). Furthermore, they were the most frequently examined quinolones, with high concentrations in China's surface water (Huang et al. 2020). Because research on antibiotics in the Huai River is limited, the concentration data were compared to those of the nearby Yangtze River. Mean concentrations of OFX, CFX, NFX, EFX in the Yangtze River were 19, 16, 25 and 20 ng/L (Li et al. 2018b). Except

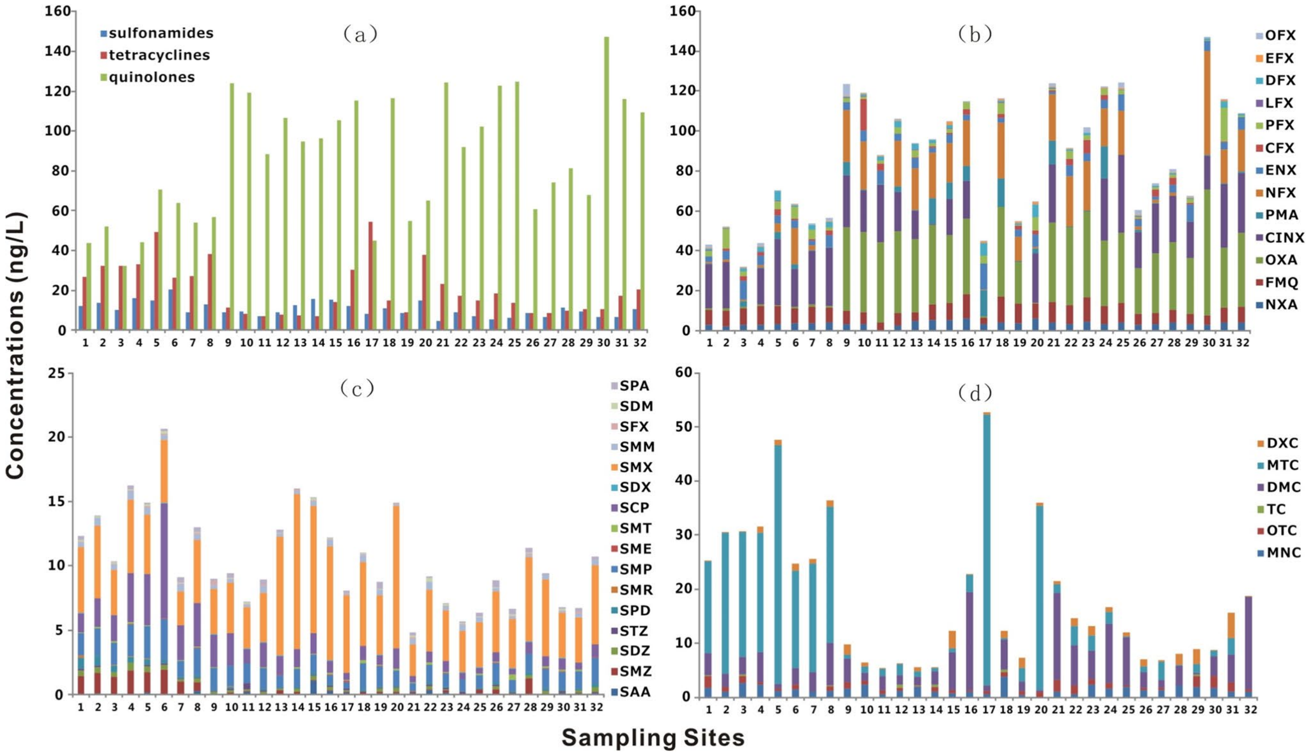

Fig. 2 Concentrations of ∑sulfonamides, ∑tetracyclines and ∑quinolones (**a**), specific quinolones (**b**), sulfonamides (**c**) and tetracyclines (**d**) at different sampling sites

for NFX, concentrations of the other three quinolones in the Huai River were about one to two orders of magnitude lower than those detected in the Yangtze River (Table 1). This could be due to the Huai River basin having a lower population density and emission intensity than the Yangtze River basin (Zhang et al. 2015).

It is worth noting that the Huai River's quinolone priority differed significantly from that of all Chinese rivers and lakes because OXA and CINX were seldom assigned as the target analytes previously (Huang et al. 2020; Li et al. 2018b, 2020b; Liu et al. 2018). OXA and CINX are first-generation quinolones that have long been utilized (Fierens et al. 2000). As "old" antibiotics, OXA and CINX are no longer utilized in human medicine. However, OXA remains on the "list of essential medicines for animals" (Ministry of Agriculture and Rural Affairs of China, announcement #2069). Since the strong antibacterial activity of OXA against fish pathogenic bacteria, such as *Vibrio anguillarum* and *Aeromonas hydrophila*, even at a low dosage rate, and low bioavailability in fishes, it has been widely used in Chinese aquaculture systems (Chen and Wang 2015; Liu et al. 2020). There is no indication that OXA was used in livestock & poultry, hence the high levels of OXA detected in the Huai River might be attributed mostly to the aquaculture industry. According to the spatial distribution of quinolones, concentrations of OXA increased abruptly in the middle and lower sections (Fig. 2b). Furthermore, NFX, which has been used in both animals and humans, exhibited a similar trend (Fig. 2b). This phenomenon might be attributed to the vigorous development of aquaculture industries in the catchment area of the middle and lower sections. Although CINX was rarely reported in China's surface water, a previous study in Xuzhou, which is located in the Huai River basin, found that CINX was the dominating antibiotic in poultry waste (Gu et al. 2019). Furthermore, CINX was frequently detected in the water of the East China Sea (Li et al. 2020a). As a result, CINX may be still frequently utilized in veterinary medications in China.

Sulfonamides

The primary sulfonamides in the Huai River were SMX, SCP, and SMP, with mean concentrations of 5.3 ± 2.4, 1.7 ± 1.6, and 1.5 ± 0.58 ng L^{-1}, respectively. They contributed 68%–93% of aqueous ∑sulfonamides (mean = 80%, Fig. 2c). Mean concentrations of other sulfonamides were < 0.48 ng L^{-1} (Table 1).

Similar to the result of this study, a lot of previous studies also found that SMX was the most commonly detected and the dominant sulfonamide in China's surface water (Li et al. 2018b; Zhou et al. 2019). Concentrations of SMX in the Huai River (mean = 5.3 ng L^{-1}) were lower than those in the Yangtze River (mean = 35 ng L^{-1}) and Yellow River (mean = 34 ng L^{-1}), but were comparable to those in the lakes of the middle-lower-Yangtze Plain (mean = 4.2 ng L^{-1}) and Huixian karst wetland (Guilin, mean = 8.7 ng L^{-1}) (Li et al. 2018b; Qin et al. 2020; Zhou et al. 2019). The use of SMX in China in 2013 was 313 tons, accounting for only 4% of total sulfonamide consumption (Zhang et al. 2015). Although not commonly used, SMX is more persistent and resistant to biodegradation, hence it is more broadly dispersed in China's surface water (Adamek et al. 2016; Al-Ahmad et al. 1999; Jin et al. 2016).

In addition to SMX, SMZ and SDZ were also widely detected in lakes and rivers in China (Li et al. 2018b, 2020b). Concentrations of SMZ (mean = 0.48 ng L^{-1}) and SDZ (mean = 0.22 ng L^{-1}) in the Huai River were about one to three orders of magnitudes lower than those detected in the Yangtze River (means of SMZ and SDZ were 21 and 50 ng L^{-1}, respectively) and Yellow River (means of SMZ and SDZ were 13 and 1.8 ng L^{-1}, respectively) (Li et al. 2018b).

SCP and SMP were not the most often detected sulfonamides in Chinese surface water. SCP in the Huai River (mean = 1.7 ng L^{-1}) was comparable to that in the Yangtze River (mean = 4.3 ng L^{-1}) (Li et al. 2018b). SMP (mean = 1.7 ng L^{-1}) was, however, clearly lower than that in the Yangtze River (mean = 130 ng L^{-1}). Previous studies reported high levels of SMP and SCP in surface water or wastewater around livestock farms and fishponds in China (Gu et al. 2019; Luo et al. 2011). Geographically, concentrations of SCP and SMP were much higher in the upper section than in the middle and low sections (Fig. 2c). SMZ also demonstrated a similar spatial distribution. As a result, SCP, SMP, and SMZ might have some special usage (livestock & poultry farming) in the upstream area (see Section "Source identification"). SMX exhibited no such tendency in this study.

Tetracyclines

MTC and DMC were the dominant tetracyclines with concentrations of 9.8 ± 14 and 5.0 ± 4.7 ng L^{-1}, respectively. They contributed about 75% of ∑tetracyclines (Fig. 2d). MNC, OTC, and DXC were also widely detected in the Huai River, but their concentrations were lower than those of MTC and DMC. TC and its two transformation products, ETC and ATC, were rarely detected (Table 1). Previous studies in the Yangtze River found OTC and TC were the dominant tetracyclines, with concentrations reaching several thousand ng L^{-1} (mean = 160 and 210 ng L^{-1}, respectively). However, their concentrations in the Huai River were more than three orders of magnitude lower (Table 1).

Unlike TC and OTC, only a small number of studies have included MTC and DMC as targets, thus they have previously been found in only a few Chinese rivers and lakes (Li et al. 2018b, 2020b). However, some studies are showing that they could be detected at high concentrations (up to or nearly several mg L^{-1}) in animal wastewater in China (Huang et al. 2020; Jiang et al. 2013; Wan et al. 2021). In this study, MTC concentrations were found to be high at all upper-section sites, and MTC contributed 63–89% of ∑tetracyclines for these sites (Site 1–8, Fig. 2d). This might be owing to the input of MTC from livestock & poultry farming of the upstream agricultural area. Furthermore, MTC at sites 17 and 20 was much higher than at other sites in the middle region (Fig. 2d), indicating there might be fragmented farming in this region. However, the DMC trend was different from MTC; the three sites with the highest concentrations (sites 16, 21, and 32) were all found in the middle and low sections (Fig. 2d), indicating that DMC and MTC came from different sources (see Section "Source identification").

Source identification

One of the most important issues in antibiotic monitoring is source identification and apportionment. Dimensionality reduction is typically considered throughout the source identification of environmental pollutants, i.e. the transformation of complex concentration data to low-dimensional representations that retain some meaningful properties of the original data while also representing the sources of these pollutants (Wu et al. 2019; Yuan et al. 2021). In this study, correlation analysis and positive matrix factorization (PMF) were combined to analyse the correlations between antibiotics and investigate the possible sources of the observed antibiotics.

According to the antibiotic correlation matrix, there was a strong positive correlation between OXA and NFX ($R=0.77$, $P<0.01$), indicating that these two priority quinolones had common origins, such as aquaculture industries (Fig. 3). CINX, another priority quinolone, however, showed no relationships with OXA or NFX. Most sulfonamides, including SCP, SMP, SPD, SMZ, and SDZ, as well as MTC, were shown to have negative correlations with OXA (Fig. 3). These findings suggested that their application fields might be significantly different. Because OXA was primarily used in aquaculture (see Section"Antibiotics in surface water of Huai River"), non-aquaculture applications might be the primary sources of MTC and the abovementioned sulfonamides. For the three priority sulfonamides, SMP and SCP had a very significant positive connection ($R=0.80$, $P<0.001$), whereas SMX had no correlations with SMP or SCP ($R=-0.03$ and -0.02). Furthermore, sulfonamides with relatively high mean concentrations, such as SMZ, SDZ, SPD, and SMM, showed significant ($R>0.6$) or moderate ($R>0.4$) positive correlations with SMP and SCP ($P<0.05$), showing that most sulfonamides came from similar sources. Most tetracyclines exhibited no relationships with one other or with other antibiotics.

For PMF model analysis, 2–6 factors were tested to explore the most application results with a running number of 30 (random start). The model parameters, such as the uncertainty-scaled residuals and correlation coefficients between observed

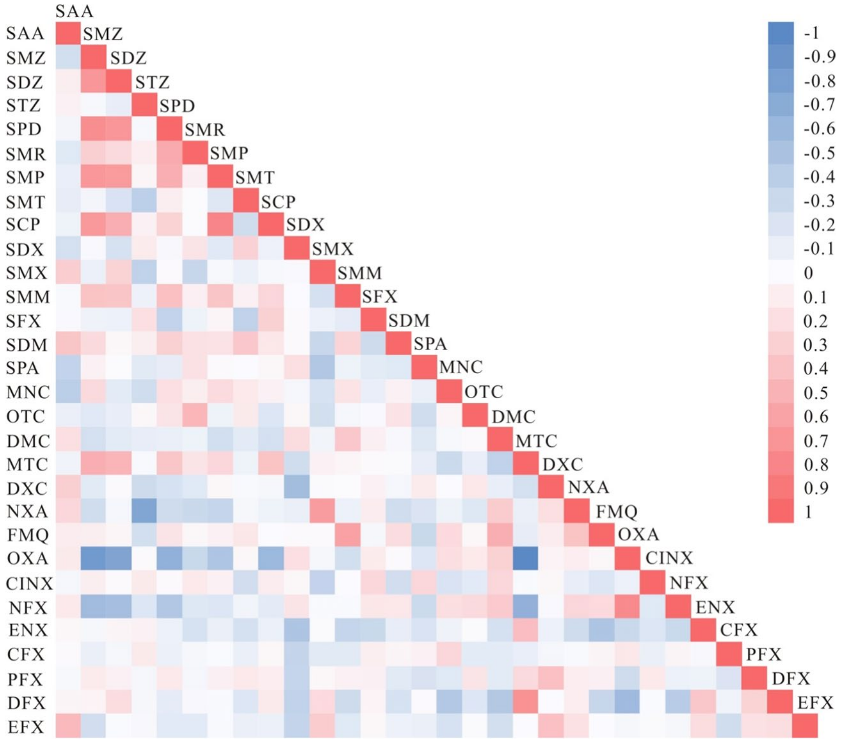

Fig. 3 Heatmap of correlations between different antibiotics in the Huai River

and anticipated values, were gradually optimized based on the test run results. Finally, the number of the factors was determined to be 3 (Fig. 4, details of the base run statistics were presented in Table S6). The results of PMF indicated only some predominant antibiotics (they were set as "strong" species) showed good correlations between observed & predicted values, such as SMP ($r^2=0.62$), SCP ($r^2=0.50$), MTC ($r^2=0.81$), DMC ($r^2=0.26$) and OXA ($r^2=0.70$). However, there were still some predominant antibiotics that did not exhibit this feature, including SMX ($r^2=0.02$) and CINX ($r^2<0.01$). According to the PMF results, factor 1 was dominated by a number of quinolones, such as NFX, OXA, and MFQ, which are primarily utilized in aquaculture industries (see Section"Antibiotics in surface water of Huai River" and Fig. 3) (Chen and Wang 2015; Liu et al. 2020). Factor 2 was dominated by most sulfonamide, including SCP, SMP, SMZ, SDZ, SPD, as well as MTC, which are mainly derived from livestock & poultry farming (Chinese Veterinary Pharmacopoeia Committee 2020; Gu et al. 2019; Zhang et al. 2015). The composition of factor 3 was complex, EFX, CINX, DXC, and SDZ were the antibiotics with high contributions. According to the veterinary pharmacopeia of China, EFX is only used in animals (Chinese Veterinary Pharmacopoeia Committee 2020). CINX was not included in the veterinary pharmacopeia, however, it has been widely detected in soil, wastewater, and groundwater of livestock & poultry farm in China (Chinese Veterinary Pharmacopoeia Committee 2020; Gu et al. 2019). DXC and SDZ could be easily bought online and were utilized in both animals (including livestock, pets, and fishes) and people (Figure S1). As a result, factor 3 was determined to be mixed sources. To summarize, the aquaculture industry was the major source of antibiotics (49%) followed by livestock & poultry farming (26%) and mixed sources (26%). As shown in Fig. 4b, antibiotics in the upper section were mainly influenced by livestock & poultry farming, while the aquaculture industry and mixed sources predominated in the middle and lower sections. This regional variance might be attributed to changes in industry, hydrological, and demographics along the river. According to the provincial land-use plan (2006–2020, issued by the the people's government of Anhui Province) and rural industry development plan (2021–2025, issued by department of agriculture and rural affairs of Anhui Province), the upper reaches

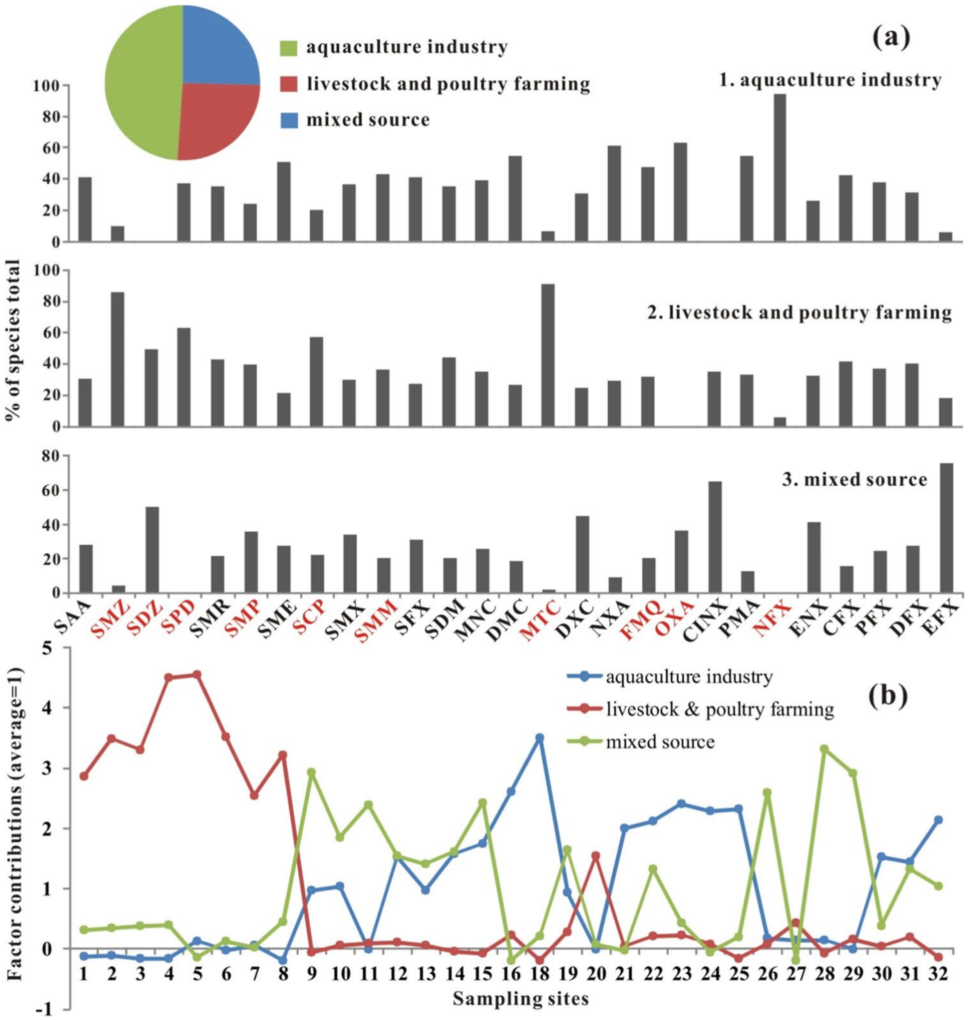

Fig. 4 (**a**) Factor profiles of the individual sources (bar chart) and relative contribution of each factor to the total antibiotics (pie chart), antibiotics in the red text means it showed good correlations between observed & predicted values ($r^2>4.2$); (**b**) Source factor contributions at different sampling sites

are primarily agricultural areas, with livestock & poultry farming being well-developed. The middle and lower reaches are rich in water resources and are more suitable for the development of aquaculture. Furthermore, two regional central cities with millions of people, i.e., Huainan and Bengbu, are located in this region, thus, the human-use antibiotic may be also an important antibiotic source.

Results of ecological risk assessment

In this study, *Green algae* and *Daphnia magna* were chosen to reflect the aquatic toxicity, since they are commonly used in aquatic toxicity testing, and relevant toxicity data are relatively abundant. The assessment factor was set as 1000 because acute toxicity data were used here (Table S7) (Qin et al. 2020). The results of the ecological risk assessment were presented in Figure S2. Generally, risk levels can be classified into: negligible ($RQ < 0.01$), low ($0.01 \leq RQ < 0.1$), medium ($0.1 \leq RQ < 1$), and high ($RQ \geq 1$) (Chen et al. 2022; Qin et al. 2020). For *Green algae*, almost all the RQs of individual antibiotics were < 0.01, suggesting negligible ecological risks. However, the values of RQ_{mix} were in the range of 0.01–0.24, indicating the low risks for *Green algae*. Although individual antibiotics presented low risks for *Green algae*, the combined effect of antibiotics might lead to a considerable integrated risk (Eguchi et al. 2004). For *Daphnia magna*, 20/32 and 5/32 samples had the RQs of CINX in the ranges of 0.01–0.1 (low risk) and 0.1–1 (medium risk), respectively. Furthermore, the RQ values of all the other antibiotics were < 0.01, except for one sample (site 10) which had RQ of CFX in the range of 0.01–0.1 (low risk). Furthermore, RQ_{mix} of 9/32 samples fell within the range of 0.1–1, and 21/32 samples fell within the range of 0.01–0.1. That is to say, most samples presented medium or low integrated risks for *Daphnia magna*, and CINX deserved some attention. Because testing toxicity data for other aquatic creatures, such as fishes, were lacking, only two species (phytoplankton and zooplankter) were employed for the ecological risk assessment. Further toxicological studies are still needed to illuminate the risks of antibiotics to aquatic ecosystems. In addition, some recent studies have suggested that antibiotics may drive resistance selection in bacteria, even at low concentrations (Murray et al. 2021). However, toxicological data related to antimicrobial resistance is still lacking, and more research is needed in order to fully reveal the environmental risks of antibiotics.

Conclusion and enlightenment

Three types of widely used antibiotics, quinolones, sulfonamides, and tetracyclines, were analyzed in the Huai River, a major Chinese River, and concentrations of $\sum$quinolones (86 ± 31 ng/L) > $\sum$tetracyclines (20 ± 13 ng/L) > $\sum$sulfonamides (11 ± 3.7 ng/L). The aquaculture industry was determined in this study to be the major source of the observed antibiotics (49%), followed by livestock & poultry farming (26%) and mixed source (25%). Antibiotics in the upper section were mostly impacted by livestock & poultry farming, but in the middle and lower sections, the aquaculture industry and mixed sources predominated. This study indicated that some rarely reported antibiotics might be the priority contaminants and deserve more extensive investigation in surface water, such as OXA, CINX, NFX, and MTC, as well as some other antibiotics with relatively high concentrations, such as SCP, SMP, and DMC. Ecological risk assessment suggested that antibiotics posed negligible or low integrated risks for *Green algae*, and medium or low integrated risks for *Daphnia magna*.

According to China's current relevant environmental laws, regulations, and policies, livestock & poultry farming waste is absolutely forbidden from entering the water body directly. Antibiotics used in livestock & poultry farming, however, can still be discharged into the soil and water environment during and/or after treatment (composting, retting et, al.). Antibiotics used in aquaculture are easier to enter water bodies than antibiotics used in livestock & poultry farming because aquaculture water is usually connected directly with rivers and lakes. Commercial fishing has been prohibited in most of China's main rivers and lakes to protect freshwater biodiversity, including the Huai and Yangtze rivers. However, consumer demand for aquatic products remains high in China. As a result, it is expected that the aquaculture industry will expand in the next years. Antibiotic contamination caused by the aquaculture industry will become increasingly serious, and relatively studies and control strategies still need to be improved.

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Data availability All data generated or analyzed during this study are included in this published article and its supplementary information files.

Declarations

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Consent to participate Not applicable

Consent to publish Not applicable

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