Distribution of Sulfonamide Antibiotics and Resistance Genes and Their Correlation with Water Quality in Urban Rivers(Changchun City), China in Autumn and Winter

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Research Article

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Abstract

With the extensive use of antibiotics, antibiotics contamination and antibiotic resistance genes (ARGs) in the water environment is becoming severe. In this study, the distribution characteristics of sulfonamide antibiotics and resistance genes in the urban section of the Yitong River in autumn and winter were studied. The correlation between them and water quality parameters was analyzed using liquid chromatography/mass spectrometry (LC/MS/MS) and polymerase chain reaction (PCR) fluorescence quantitative technology. The results showed that the concentration of seven sulfonamide antibiotics in surface water and sediment of the river was generally at the level of ng/L and ng/g; the total concentration range was 11.08-160.60ng/L and ND-85.68ng/g, respectively. The total concentrations of antibiotics were similar in autumn and winter, where SMX was the primary type of antibiotics. The results of Risk Quotients (RQs) showed that SMX and SDZ had moderate acute risk to the corresponding sensitive species in river water, SPD and SIZ had low acute risk, while the rest had no risk. The total bacterial abundance in surface water and sediment was in the range of 10^5 - 10^6 and 10^8 - 10^{11} , respectively. The detection rates of three sulfonamide resistance genes were 100%; the relative abundance was in the range of 10^{-3} - 10^{-1} , and *su1* was the primary resistance gene. The correlation analysis showed that most antibiotics were significantly related. The three resistance genes had a positive correlation, negative correlation, and non-correlation, and most antibiotics had a weak correlation with resistance genes. Nutrients had a more significant impact on antibiotics, and other water quality indicators had a more negligible effect on antibiotics and resistance genes.

Introduction

In recent years, with the extensive use of antibiotics, the water environment antibiotics and resistance genes pollution are increasingly severe, posing a threat to human and aquatic ecological security. Antibiotics are a class of organic substances that are mainly produced or synthesized by microorganisms and can inhibit or affect the function of microorganisms. Like drugs, they are widely used in human medical treatment, animal disease prevention and treatment, animal husbandry, aquaculture, and other fields (Xiong et al. 2015). However, more than 30% of antibiotics cannot be absorbed by bio metabolism and are released into the environment with the patient's urine and faeces (Wang et al. 2020). The correlation studies show that antibiotics have a higher detection rate in water, sediment, organisms, and other media (Chen et al. 2015; Fernandes et al. 2020; Li et al. 2012; Liang et al. 2013; Liu et al. 2019; Luo et al. 2011). Antibiotic residues cause chemical contamination in the environment and induce the generation of resistance genes. Antibiotic resistance genes, as new pollutants, can be propagated to the offspring through microorganisms and can be horizontally transferred between bacteria (Luo et al. 2010). Compared with antibiotics, they are more harmful and identified by the World Health Organization (WHO) as significant environmental problems in the 21st century (Rodriguez-Mozaz et al. 2015).

The Yitong River is an important tributary of the Songhua River. There are 48.82 kilometers of river sections flowing through the urban area and the north and south of Changchun City. The Yitong River accepts the discharge of sewage in the city area. Although part of the sewage is treated before being

discharged into the river, the sewage treatment plant is not ideal for removing antibiotics, and the river will still be polluted. There have been many studies on the contamination of antibiotics and resistance genes in the water environment. However, the contamination of antibiotics and resistance genes in the water environment of urban rivers in northern China is relatively rare, especially in the water environment of the Yitong River. The environment of the Yitong River belongs to the type of continental semi-humid monsoon climate in the northern temperate zone. The change of seasons is noticeable. Especially in autumn and winter, it has a low temperature, slight precipitation, and small runoff. Some studies have shown that the concentration of antibiotics in river water is higher in autumn and winter (Yang et al. 2011; Younes et al. 2019). Therefore, through the detection and analysis of sulfonamide antibiotics, resistance genes, and water quality indicators in the water environment of the Yitong River in autumn and winter, the distribution characteristics and correlation were studied. This provided the basis for environmental management and risk assessment of the Yitong River and provided data and reference for pollution control and ecological protection of Northern urban rivers in China.

Materials And Methods

1.1. Standard Samples and Reagent Drugs

The standard samples of seven sulfonamides antibiotics in this study were; sulfamerazine (SMZ1), sulfamethazine (SMZ), sulfamethoxazole (SMX), sulfisoxazole (SIZ), sulfathiazole (STZ), sulfadiazine (SDZ) and sulfapyridine (SPD). The standard internal substance is sulfamethoxazole-d4. All standard products were purchased from the Beijing Tanmo Quality Technology Co., Ltd-China. The purity $\geq$ was 98%.

The reagents used in the experiment were chromatographically pure, while the drugs were analytically pure. Methanol, acetonitrile, and formic acid were purchased from Thermo Fisher Technology Co., Ltd-China. Ammonia and ethylenediaminetetraacetic acid disodium (EDTA) were purchased in MACKLIN Company, and while the McIlvaine buffer solution was purchased in Shanghai Yuanmu Biological Technology Co., Ltd.-China. And the phosphate buffer solution was purchased in Biosharp Biotech Co., Ltd.-China. And the experimental water was purified water.

1.2. Study area and sample collection

This study selected the 48.82 km section of the Yitong River through the urban area of Changchun City as the research area and eight spots as sampling points. The sampling points include the Xinlicheng Reservoir, South City-round Expressway Bridge, South-third-ring South Barrier Gate, Rubber Dam of Small Slab Bridge, Free Barrier Gate, Xinghua Barrier Gate, Sihua Barrier Gate, and North Lake Bridge. The sampling point was recorded as S1-S8, and its distribution is as Fig. 1. S1 point was the source of the research area, with good water quality, few human populations, a large number of surrounding farmlands, and abundant aquatic plants, which is an important water source and flood control barrier of the city. S2,

S3-S7, and S8 points were the study's upper, middle, and lower reaches, densely populated, surrounded by many buildings, roads, parks, sluices, drainage outlets, and other facilities.

The sampling time was September and November 2020, in autumn and winter exchange seasons in the north of China. Organic glass water samplers and Peterson mud samplers were used to collect surface water and sediment samples. Three parallel samples were collected at each sampling point, and the collected samples were refrigerated in ice bags away from light and transported to the laboratory for testing.

1.3. Antibiotic detection

The pretreatment of antibiotics detection was carried out as done previously (Wang 2014). It was determined using a liquid chromatography-mass spectrometry (3200 Q TRAP LC/MS/MS system). The chromatography column was InfinityLab Poroshell 120 EC-C18(2.1×100mm, 2.7-Micron, Agilent, USA). The column was operated in the positive ion mode of electrospray ionization (ESI).

The concentration of antibiotics was determined by the standard internal method. The standard curves (10-50 µg/L) of each antibiotic showed an excellent linear relationship ($R^2 > 0.99$), which met the requirements of analysis. The recoveries of antibiotics in surface water and sediment were 77.66%-98.90% and 75.47%-95.30%, respectively. The detection limits were 0.01~0.31ng/L, and 0.01~0.15ng/g (S/N=3), and the quantitative limits were 0.03~0.72ng/L 0.03~0.45ng/g (S/N=10).

1.4. Detection of antibiotic resistance genes

1.4.1. DNA extraction

DNA extraction was carried out on the water filtration membrane, and sediment samples were stored in a -80°C refrigerator. The reagent kit (cat: M5635-02) produced by Omega Company was selected and operated strictly according to the kit's instructions.

1.4.2. PCR amplification experiment

The corresponding resistance gene (sul1, sul2, sul3) and the internal control gene (16S rRNA) of sulfa antibiotics were detected by PCR. The primers used in this study were prepared by Shanghai Personalbio Gene Technology Co., Ltd. The primers sequence, target gene fragment size, and annealing temperature were shown in table 1.

Table 1 PCR primers and reaction conditions of target gene

ARGs	Primer	Primer sequence (5' to 3')	Primer length	Annealing temperature
16S rRNA	16s-F	ATGGCTGTCGTCAGCT	337	60°C
	16s-R	ACGGGCGGTGTGTAC		
sul1	sul1-F	CGCACCGGAAACATCGCTGCAC	163	65°C
	sul1-R	TGAAGTTCCGCCGCAAGGCTCG		
sul2	sul2-F	TCCGGTGGAGGCCGGTATCTGG	191	57.5°C
	sul2-R	CGGGAATGCCATCTGCCTTGAG		
sul3	sul3-F	TCCGTTTCAGCGAATTGGTGCAG	143	61°C
	sul3-R	TTCGTTTCAGCCTTACACCAGC		

1.4.3. qPCR quantitative experiment

The PCR reaction solution configured according to the reaction system was placed on a Real-time PCR instrument for performing the PCR reaction. The reaction procedure was as follows: predenaturation at 95°C for 5min; denaturation at 95°C for 15s; elongation at 60°C for the 30s. Then, the cycle from denaturation to elongation was repeated 40 times. All samples were set up with three parallel samples. The ARG standard curve showed a good linear relationship with the correlation coefficient $R > 0.996$ and the amplification efficiency between 80.80%-94.60%, which could calculate each gene's copy number and meet the experimental requirements.

1.5. Detection of water quality parameter

The surface water quality parameters such as pH, water temperature (WT), dissolved oxygen (DO), water transparency (SD), chemical oxygen demand (COD_{Cr}), total nitrogen (TN), ammonia nitrogen ($\text{NH}_3\text{-N}$), total phosphorus (TP), and chlorophyll a (Chl-a) were determined according to the "Standard Method of Water and Wastewater Monitoring (Fourth Edition)" issued by the Ministry of environmental protection of the people's Republic of China (Ministry of environmental protection of the people's Republic of China 2002). The related detection methods are listed in Table 2.

Table 2 Water quality indicator detection methods

Water quality index	Detection method
pH	Portable pH meter
WT	Thermometer
DO	Portable DO instrument
SD	Seidel disk method
COD _{Cr}	Rapid determination of COD
TN	Potassium persulfate oxidation ultraviolet spectrophotometry
NH ₃ -N	Nessler reagent spectrophotometry
TP	Molybdenum antimony resistance Spectrophotometry
Chl-a	Ultrasonic extraction

1.6. Data analysis method

Statistical analysis of all the data was carried out using MS Excel, SPSS, and other software. In addition, the Pearson method was used for correlation analysis, and drawing charts were carried out by origin 2018.

Results And Discussion

3.1. Distribution characteristics of antibiotics in water environment

3.1.1. Distribution of antibiotics in surface water

The concentration range and detection rate of antibiotics in surface water are shown in Table 3. In September, the total concentration range was 14.45-160.60ng/L, the average concentration was 69.26ng/L, and the detection rate ranged from 13% to 100%. The concentration of SMX was the highest, the range of concentration was 5.50-77.76ng/L, the average concentration was 29.67ng/L, the detection rate was 100%. The detection rates of SMZ (13%) and SIZ (38%) were low, and the detection rates of other species were 100%. In November, the total concentration range of the detection was 11.08-109.34ng/L, the average concentration was 61.84ng/L, and the detection rate ranges from 0% to 100%. Among them, the concentration of SMX was the highest, SMZ was not detected, and the detection rate of other species was 100%. Overall, the seven sulfonamide antibiotics were seen, the concentration was at

the level of ng/L, and the total concentration was similar in autumn and winter. SMX was the primary antibiotic species, which was consistent with previous studies (Kolpin et al. 2002). Due to its unique occurrence characteristics, SMX in water samples can produce resistance among microorganisms and persist in the ecosystem (Prasannamedha et al. 2020). This section's SMX concentration in surface water was higher (Chen et al. 2002). The low detection rate of SIZ in September and the high rate in November may be due to a source of such antibiotics in the vicinity of the river, which should be taken seriously.

Table 3 Antibiotic concentrations in surface water of the Yitong River

	Antibiotics	Range∅ng/L∅	Mean∅ng/L∅	Freq∅%∅
September∅n=8, ng/L∅	SMZ1	1.10-4.03	2.50	100%
	SMZ	ND-0.36	0.05	13%
	SMX	5.50-77.76	29.67	100%
	SIZ	ND-2.64	0.73	38%
	STZ	1.38-10.32	5.89	100%
	SDZ	5.78-36.73	14.50	100%
	SPD	0.69-28.76	15.92	100%
November∅n=8, ng/L∅	SMZ1	1.91-4.88	3.54	100%
	SMZ	ND-ND	0.00	0%
	SMX	5.98-36.31	20.28	100%
	SIZ	ND-13.16	9.27	100%
	STZ	1.49-6.78	3.90	100%
	SDZ	1.36-22.80	11.61	100%
	SPD	0.34-25.41	13.24	100%

Note: ND means not detected.

The spatial distribution of antibiotic concentration in the surface water is shown in Fig. 2. The total concentration of the S7 site was the highest in September, which was 149.98ng/L. The concentrations of the S3-S6 sites were high, the range was 81.35-90.30ng/L, and the rest were low, ranging from 16.06 to 23.64ng/L. In November, the total concentration of the S7 site was the highest (97.94ng/L), S4-S6 sites concentrations were high, the range was 73.81-89.37ng/L, the rest were low, ranging from 22.87 to 52.42ng/L. Generally speaking, the total concentration of S1, S2, S8 points were low; meanwhile, the total concentration of S3-S7 point was high, which may be related to the surrounding environment of the river section. The surrounding of S3-S7 point is densely populated and has a sewage outlet. While S1 is the outlet of Xinlicheng Reservoir, S2 and S8 are located around parks with a good environment. SMX, SDZ,

and SPD concentrations were high, which were the main antibiotics in the reach, indicating that the three antibiotics were primarily used in the surrounding environment. However, the concentration of SIZ at each site was significantly high in November, meaning there was a potential source of this antibiotic around the area.

3.1.2. Distribution of antibiotics in surface sediments

The concentration range and detection rate of antibiotics in surface sediments are shown in Table 4. In September, the total concentration ranged from 3.36 to 85.68ng/g, the average concentration was 18.78ng/g, and the detection rate ranged from 25% to 100%. SMX was the main component in surface sediment, the same as the main antibiotics in surface water. The concentration ranged from 3.01 to 54.20ng/g, the average concentration was 12.41ng/g, and the detection rate was 100%. Except for SMZ1 and SPD, the detection rates of other species were lower. The total concentration range of November was ND-24.31ng/g, the average concentration was 15.17ng/g, and the detection rate ranged from 0% to 88%, among which the concentration of SIZ was the highest, meanwhile, SMZ and STZ were not detected. Overall, the seven sulfonamides antibiotics were all detected, the concentration was at the level of ng/g, and the total concentration in autumn and winter was similar. Same as surface water, SMX was the primary type of antibiotic. SMX and SDZ had higher concentrations than other parallel rivers.

The spatial distribution of antibiotic concentrations in surface sediments is shown in Fig. 3. In September, the total concentration of the S1 site was the highest, thus 70.60ng/g, S3 site was the higher concentration, 40.21ng/g. The rest of the site concentration was low, and the range was 4.06-9.70 ng/g. In November, the concentration of each point was low; the range was ND-21.73ng/g. Generally speaking, SMX was the main antibiotic in September, and SIZ was dominant in November, respectively. Different levels of antibiotic pollution can reflect the characteristics of antibiotic use and discharge in the surrounding area of the urban river. The results in Fig. 3 show that the antibiotic concentration of most points was low, which indicated that the antibiotic pollution in the surface sediment of this section was relatively light. However, the potential sources of various antibiotics still need to be paid attention to.

Table 4 Concentration of antibiotics in surface sediments of the Yitong River

	Antibiotics	Range (ng/g)	Mean (ng/g)	Freq (%)
September (n=8 ng/g)	SMZ1	0.31-3.62	0.92	100%
	SMZ	ND-14.73	2.72	25%
	SMX	3.01-54.20	12.41	100%
	SIZ	ND-8.23	1.46	50%
	STZ	ND-1.49	0.37	38%
	SDZ	ND-2.38	0.54	38%
	SPD	0.04-1.03	0.36	100%
November (n=8 ng/g)	SMZ1	ND-2.12	1.00	88%
	SMZ	ND-ND	0.00	0%
	SMX	ND-3.70	2.46	88%
	SIZ	ND-12.48	9.41	88%
	STZ	ND-ND	0.00	0%
	SDZ	ND-3.71	1.39	75%
	SPD	ND-2.30	0.91	88%

3.1.3. Ecological risk assessment of antibiotics

The Risk Quotients method proposed by Hernand *et al.* was used to evaluate the risk of antibiotics (Hernando et al. 2006). According to the EU standards, the specific calculation formula is as follows (Garnier-Laplace et al. 2008):

$$RQs = MEC / PNEC \times 10^1$$

In the formula: MEC is the measured environmental concentration (ng/L), considering the worst risk situation, choose the maximum concentration as the risk evaluation value; PNEC is the predicted no-effect concentration (ng/L).

$$PNEC = NOEC / AF = EC50 / AF \quad (2)$$

NOEC is the no observed effect concentration of the most sensitive species (mg/L); EC50 is the concentration for 50% of maximal effect (mg/L); AF is the assessment factor.

Acute toxicity data were used in this study, where AF was 1000 (Park and Choi 2008). The value of PNEC was obtained using the EC50 toxicity data obtained in the literature; details are shown in Table 5 (Białk-Bielińska et al. 2011). In addition, according to the RQs classification method proposed by Hernando *et al.*, the risk assessment was divided into four levels: no risk (<0.01), low risk (0.01-0.1), medium risk (0.1-1), and high risk (>1) (Chen et al. 2020).

Table 5 Toxicological data of antibiotics for sensitive species

Antibiotics	Species	Toxic type	Evaluation factors	EC50(mg/L)	PNEC(ng/L)
SMZ1	Lemna minor	acute	1000	0.68	680
SMZ	Lemna minor	acute	1000	1.74	1740
SMX	Lemna minor	acute	1000	0.21	210
SIZ	Lemna minor	acute	1000	0.62	620
STZ	Lemna minor	acute	1000	4.89	4890
SDZ	Lemna minor	acute	1000	0.07	70
SPD	Lemna minor	acute	1000	0.46	460

Fig. 4 shows the RQs of the target antibiotics in surface water in autumn and winter. The results showed that SMX and SDZ showed the moderate acute risk to the sensitive species in river water in September, SPD showed low acute risk, and others showed no risk. In November, SMX and SDZ showed moderate acute risk, SIZ and SPD showed low acute risk, and others showed no risk. Generally, there was a specific problem of antibiotic pollution in the water environment of the target river, but it had not reached the high risk. Therefore, the control and management should be strengthened to reduce the ecological risk.

3.2. Distribution characteristics of resistance genes in water environment

Three sulfonamide resistance genes and 16S rRNA internal control genes in water environment samples were detected in the autumn and winter. The relative quantitative analysis of target gene abundance distribution was used to avoid the difference caused by DNA extraction efficiency and environmental microbial background value (Yang et al. 2016). The sampling points were S3, S5, and S7, and the sample names were NW9, ZW9, SW9, NW11, ZW11, and SW11.

3.2.1. Distribution of resistance genes in surface water

The absolute abundance of 16S rRNA in the surface water is shown in Fig. 5. The absolute abundance range was 1.92×10^5 - 1.29×10^6 copies/ μ L in September and 1.87×10^5 - 2.57×10^5 copies/ μ L in November. In general, the total bacterial abundance was similar in autumn and winter, in the range of 10^5 - 10^6 . The relative abundance of that three sulfonamide resistance genes is shown in Fig. 6. The detection rate was 100% in autumn and winter, and the total relative abundance range was 1.43×10^{-2} - 9.98×10^{-2} copies/16S rRNA in September and 5.91×10^{-2} - 1.19×10^{-1} copies/16S rRNA in November. Overall, sul1 was the main resistance gene in September, and sul2 was the main resistance gene in November. The total relative abundance was similar in the 10^{-2} - 10^{-1} order of magnitude. In terms of temporal distribution, the relative abundance of resistance genes was November (2.63×10^{-1} copies/16S rRNA) > September (1.50×10^{-1} copies/16S rRNA). Again, in terms of spatial distribution, the relative abundance of sites ranged from 10^{-3} - 10^{-2} orders of magnitude in September to 10^{-3} - 10^{-1} orders of magnitude in November. Overall, the relative abundance of each site in autumn and winter was sul2 (2.51×10^{-1} copies/16S rRNA) > sul1 (1.31×10^{-1} copies/16S rRNA) > sul3 (3.23×10^{-2} copies/16S rRNA). The high detection rate and high abundance level of ARGs indicated that the widespread use of the corresponding types of antibiotics around the river and other human activities and pollution source distribution had caused some environmental risks in the region (Yang et al. 2017a). There were many people around these three sites and drainage ports, hospitals, and other construction facilities. Therefore, they received sewage treatment plants, hospitals, and domestic sewage discharge, resulting in strong ARGs pollution.

3.2.2. Distribution of resistance genes in surface sediments

The absolute abundance of 16S rRNA in surface sediments is shown in Fig. 7. The absolute abundance range of 16S rRNA was 7.45×10^9 - 1.45×10^{11} copies/g in September and 6.83×10^8 - 3.48×10^{10} copies/g in November. The difference in bacterial abundance between autumn and winter was large, in the range of 10^8 - 10^{11} . The relative abundance of that three sulfonamide resistance genes is shown in Fig. 8. The detection rate was 100% in autumn and winter, and the total relative abundance range was 1.03×10^{-2} - 6.02×10^{-2} copies/16S rRNA in September and 5.00×10^{-3} - 5.27×10^{-2} copies/16S rRNA in November. In general, sul1 was the main resistance gene in autumn and winter, and the relative abundance was similar in the range of 10^{-3} - 10^{-2} . Based on temporal distribution, the relative abundance of resistance genes in September (1.13×10^{-1} copies/16S rRNA) > November (9.59×10^{-2} copies/16S rRNA). In autumn and winter, sul1 was the main resistance gene, and the relative abundance was in the range of 10^{-2} - 10^{-1} . In the spatial distribution, the relative abundance of each point in autumn and winter was in the range of 10^{-5} - 10^{-2} orders of magnitude. Overall, the relative abundance of each site was sul1 (1.70×10^{-1} copies/16S rRNA) > sul2 (3.84×10^{-2} copies/16S rRNA) > sul3 (3.44×10^{-4} copies/16S rRNA). The high detection rate and high abundance level of ARGs indicated that the resistance gene pollution in the target river was relatively serious. A similar trend was seen in the survey results of resistance genes in surface sediments of many

lakes and rivers in the Haihe River Basin and the middle and lower reaches of the Yangtze River. Therefore, indicating that surface sediments of many lakes and rivers in China have become an important reservoir of resistance genes (Luo et al. 2010; Yang et al. 2017b).

3.3. Correlation analysis between antibiotic and resistance gene

As the antibiotics and resistance genes reservoir, the water environment plays an essential role in their storage and transmission. Studies have found a correlation between antibiotics and resistance genes (Guo et al. 2018). The Pearson correlation analysis was used to analyze the correlation between antibiotic concentration and the abundance of the corresponding resistance genes, as shown in Fig. 9 and Fig. 10. In surface water, sul1 was significantly negatively correlated with sul2 and positively correlated with sul3, while sul2 was significantly positively correlated with sul3. sul1 and sul3 were significantly positively correlated with SMZ and STZ, sul2 was significantly positively correlated with SIZ and was weakly correlated with other antibiotics. This, thus, indicated that SMZ, STZ, and SIZ promoted the formation of sul1, sul3, and sul2, respectively. SMZ was significantly positively correlated with STZ, and SMZ1 was significantly positively correlated with SPD, which indicated that they had homology with each other. SIZ was significantly negatively correlated with SMZ, STZ, and SPD, while the other antibiotics showed a weak correlation. In the surface sediments, sul1 and sul2 showed a significant positive correlation, which indicated that sul1 and sul2 had some homology.

Meanwhile, sul1 was weakly correlated with all the antibiotics tested, indicating other main influencing factors, such as environmental factors or pollutants, of resistance gene generation besides corresponding antibiotics in the water environment. sul3 was significantly negatively correlated with SMZ, SMX, and SMZ1, indicating that these antibiotics inhibited the production of sul3. On the other hand, SDZ was significantly positively correlated with SPD and significantly negatively correlated with most antibiotics. In addition, there was a significant correlation between most of the antibiotics, which indicated that they influenced each other immensely. Therefore, it could provide some reference for controlling and managing environmental pollution of sulfonamide antibiotics in urban rivers.

3.4. Correlation between antibiotic resistance genes and water quality parameter

The distribution of antibiotic resistance genes in the water environment is affected by antibiotics and is related to the water quality parameter (Li et al. 2018). Table 6 shows the test data of water quality parameter of the Yitong River. Furthermore, the correlation analysis results between water quality

parameters and antibiotic concentration and resistance gene abundance are shown in Table 7 and Table 8.

Table 6 Water quality parameter data

	Index	Min	Max	Mean
September	pH	7.239	8.404	7.765
	WT(°C)	11.9	19.2	15.6
	DO(mg/L)	6.20	12.60	9.86
	SD(m)	0.15	1.02	0.48
	COD _{Cr} (mg/L)	8.28	48.91	24.46
	TN(mg/L)	1.52	6.71	3.52
	NH ₃ -N(mg/L)	0.60	3.58	1.04
	TP(mg/L)	0.12	0.78	0.31
	Chl-a(mg/L)	0.18	4.42	2.20
November	pH	7.791	9.116	8.125
	WT(°C)	0.0	4.0	1.8
	DO(mg/L)	4.41	13.16	9.38
	SD(m)	0.10	0.75	0.34
	COD _{Cr} (mg/L)	24.83	74.50	46.18
	TN(mg/L)	0.47	2.32	0.94
	NH ₃ -N(mg/L)	0.09	0.33	0.15
	TP(mg/L)	0.15	0.51	0.27
	Chl-a(mg/L)	0.09	0.57	0.35

Table 7 shows that pH had a weak correlation with antibiotics; WT, DO, SD, and COD_{Cr} strongly correlated with some antibiotics. TN was significantly positively correlated with SMX, STZ, and SDZ and significantly negatively correlated with SIZ. NH₃-N was significantly positively correlated with SMX and SDZ; TP was significantly positively correlated with SMX and SDZ. Chl-a was significantly positively correlated with SMX, STZ, and SDZ and significantly negatively correlated with SIZ. Therefore, it was concluded that the nutrient greatly affected the antibiotics in the water environment. Table 8 shows that sul1 was not associated with all the water quality parameters; sul2 was significantly positively correlated with pH and COD_{Cr}, and significantly negatively correlated with WT and TN. sul3 was significantly

negatively correlated with pH and COD_{Cr}. In addition, the correlation between water quality parameters and resistance genes was weak, which indicated that water quality parameters had little effect on resistance genes in the water environment of this section of the Yitong River.

Table 7 Correlation between water quality parameter and antibiotic concentration

	SMZ1	SMZ	SMX	SIZ	STZ	SDZ	SPD
pH	0.106	-0.256	-0.071	0.448	-0.349	-0.343	-0.041
WT(°C)	-0.323	0.301	0.335	-0.862**	0.541*	0.222	0.288
DO(mg/L)	0.477	0.300	0.382	0.067	0.433	0.364	0.590*
SD(m)	0.285	0.066	0.554*	-0.228	0.778**	0.465	0.551*
COD _{Cr} (mg/L)	0.418	-0.188	0.391	0.596*	0.010	0.420	0.430
TN(mg/L)	-0.061	0.313	0.717**	-0.679**	0.622*	0.659**	0.481
NH ₃ -N(mg/L)	-0.103	0.049	0.697**	-0.464	0.239	0.729**	0.236
TP(mg/L)	0.045	0.045	0.707**	-0.076	0.169	0.788**	0.394
Chl-a(mg/L)	-0.165	-0.003	0.765**	-0.579*	0.532*	0.588*	0.478

Note: * indicates significant correlation at the level of 0.05, and ** indicates significant correlation at the level of 0.01.

Table 8 Correlation between water quality parameter and resistance genes abundance

	sul1	sul2	sul3
pH	-0.524	0.827*	-0.920**
WT(°C)	0.551	-0.950**	0.734
DO(mg/L)	0.394	-0.021	-0.176
SD(m)	-0.262	-0.553	0.250
COD _{Cr} (mg/L)	-0.684	0.869*	-0.870*
TN(mg/L)	0.430	-0.850*	0.403
NH ₃ -N(mg/L)	0.145	-0.594	-0.123
TP(mg/L)	-0.026	-0.129	-0.361
Chl-a(mg/L)	-0.015	-0.784	0.228

Note: * indicates significant correlation at the level of 0.05, and ** indicates significant correlation at the level of 0.01.

Conclusion

In the urban area of Changchun, the Yitong River, it has been widely polluted by antibiotics. Seven kinds of sulfonamide antibiotics were detected in surface water and sediment; the concentration was generally at the level of ng/L and ng/g. The total concentration range was 11.08-160.60ng/L and ND-85.68ng/g, among which SMX was the primary antibiotic. In autumn and winter, the total concentration of antibiotics was similar, and the pollution was more severe in the dense area of human activity. RQs showed that SMX and SDZ in surface water showed moderate acute risk to sensitive species in river water in autumn and winter, while SPD and SIZ showed low acute risk and others showed no risk. Generally, there were some problems of antibiotic pollution in the water environment of the target river section. Still, the high risks were not reached, so the control and management should be strengthened to reduce the ecological risks. In autumn and winter, the total bacterial abundance in surface water and sediment was in the range of 10^5 - 10^6 and 10^8 - 10^{11} , respectively. The detection rate of all three sulfonamide resistance genes was 100%, the relative abundance was in the order of 10^{-3} - 10^{-1} , and sul1 was the primary resistance gene. The results were similar to other watersheds in China, which indicated that the water environment of many watersheds in China had become an essential reservoir for resistance genes.

The correlation analysis results showed a positive, negative, and no correlation among the three resistance genes. Most antibiotics had a weak correlation with resistance genes, indicating other main influencing factors, such as environmental factors or pollutants, of resistance gene generation besides corresponding antibiotics in the water environment. On the other hand, most antibiotics showed a significant positive correlation, which stated that they were homologous and provided some reference for controlling and managing antibiotic pollution in urban rivers. In addition, nutrients have a more significant impact on antibiotics, while other water quality indicators have a more negligible effect on antibiotics and resistance genes.

Declarations

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Data availability The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Figures

Figure 1

Distribution of sampling points in the Yitong River

Figure 2

The concentration of antibiotics in surface water of the Yitong River

Figure 3

The concentration of antibiotics in surface sediments of the Yitong River

Figure 4

Target antibiotics RQs in surface water

Figure 5

Absolute abundance of 16S rRNA in surface water

Figure 6

Relative abundance of resistance genes in surface water

Figure 7

Absolute 16S rRNA abundance in surface sediments

Figure 8

Relative abundance of resistance genes in surface sediments

Figure 9

Correlation between antibiotics and resistance genes in surface water

Figure 10