Occurrence and influencing factors of antibiotics and antibiotic resistance genes in sediments of the largest multi-habitat lakes in Northern China

Tongfei Wang · Weijun Zhang · Guiying Liao · Meiyi Zhang · Liqing Li · Dongsheng Wang

Received: 13 April 2022 / Accepted: 22 August 2022 / Published online: 3 September 2022
© The Author(s), under exclusive licence to Springer Nature B.V. 2022

Abstract Baiyangdian Lake is a typical and largest multi-habitat lake in the North plain of China. To understand the generation and transmission of antibiotics resistance genes (ARGs) in multi-habitat lakes, the contents of nutrients (TC, TOC, TN, TP and TS), heavy metals (Zn, Cr, Ni, Cu, Pb, As, Cd and Hg), 22 antibiotics, 16S-rRNA (16S), Class I integron (intI1) and 20 ARGs were determined. Samples were taken from the Fuhe river, river estuaries, reed marshes, living area, fish ponds and open water of Baiyangdian Lake. The results showed that quinolones were the main pollutants in six habitats, and the content range was ND-104.94 ng/g. Thereinto, aac (6')-IB, blaTEM-1, ermF, qnrA, qnrD, tetG, sul1, sul2 and tetM were detected in all the analyzed samples. The absolute abundance of sul1 was the highest (5.25 × 10^5 copies/g-6.21 × 10^7 copies/g) in most of the samples. In these different habitats, the abundance of antibiotics and ARGs in river estuary was the highest, and that in reed marshes was the lowest. There was a significant positive correlation between the abundance of heavy metals (Cu, Pb, Zn, Ni, Cd, Hg) and the absolute abundance of 11 ARGs (P < 0.01). Redundancy analysis showed that Cu, Zn, intI1, TP and macrolides were the important factors affecting the distribution of ARGs. Our finding provides a more likely driving and influencing factor for the transmission of ARGs in lakes with complex and diverse habitats.

Keywords Baiyangdian lake · Sediment · Antibiotic · Antibiotic resistance gene · Influencing factor

Introduction

Antibiotics is widely used to treat the infectious diseases of human and animal by killing or inhibiting bacterial growth (Zhu et al., 2020). Recent surveys show that global antibiotic consumption is estimated to be 84 billion defined daily doses in 2030, double the amount in 2015 (Klein et al., 2018). However,
Antibiotic misuse, poor stewardship and unintentionally discharged into the environment pose a huge threat to the ecosystem and human health (Kümmerer, 2009). Over the past few decades, antibiotics have been found in environmental media such as wastewater treatment plants (WWTP) (Cheng et al., 2014b; Zhang et al., 2021), rivers (Zhang et al., 2020b), oceans (Du et al., 2017), sediments (Chen et al., 2019b; Li et al., 2018a), soil (Quaik et al., 2019), organism (Zhao et al., 2015), air (Li et al., 2018b), due to its pseudo-persistence and low biodegradability (Chen et al., 2018; Gulkowska et al., 2008). The most worrying thing is that bacteria in water and sediment are exposed to the water environment with the accumulation of antibiotics for a long time, which could be the most important contribution to increase the abundance of ARGs. (Larson et al., 2007; Luo et al., 2010; Zheng et al., 2018).

Once the bacteria mutate or acquire ARGs, they may exist in large numbers in the environment persistently, and can accelerate or even induce the production and spread of antibiotic resistance genes through horizontal gene transfer (Gillings et al., 2014; Zhu et al., 2013). Seriously, ARGs can enter the human body through the food chain, leading to the weakened or ineffective antibiotic drugs (Reichert et al., 2021). ARGs, as a new type of pollutant, has the characteristics of strong persistence, wide range of diffusion and strong fluidity, which may pose potential risks to global human health (Amarasiri et al., 2020).

In recent years, ARGs in aquatic environments have been frequently detected in different areas, such as the USA (Pruden et al., 2012), European countries (Knapp et al., 2010; Czekalski et al., 2015; Paulus et al., 2020), Australia (Han et al., 2016), Korean (Kim et al., 2017), Japan (Nguyen et al., 2019), China (Bai et al., 2021; Dong., 2021; Zhang et al., 2015), even in polar environments (Mccann et al., 2019). In particular, the pollution of ARGs in rivers or lakes in China may be more extensive and serious. For instance, the absolute abundance of sul1, tetA and tetE genes were found in seven urban rivers in Beijing (Xu et al., 2016); sulfonamide resistance genes (sul1 and sul2) were widely distributed in the water of Fuxian Lake, the deepest freshwater lake in China(Zhao et al., 2020); 321 ARGs were detected in the sediments of Tai Lake, and its abundance and risk are higher than other global lakes (Chen et al., 2019a); 15 lakes from the Yangtze river were suffered heavy sul and tet ARGs pollution (Yang et al., 2017).

However, previous work was focus on the distribution of antibiotics and ARGs in the sediments of lakes in a single habitat, and till now, their correlation or traceability has not come to a uniform conclusion in detail, especially for the lakes with different habitats. Therefore, it is extremely urgent to carry out researches to explain autochthonous antibiotics and ARGs diversity as well as to find the possible driving factors of ARG in local sediments in different habitats.

Baiyangdian Lake is an important water resource located in the Xiongan New Area of China, which is known as “the lung of North China.” And it is the largest multi-habitat lake in the North plain of China. The lake has a unique structure consisting of 143 lakes, each of them is separated and connected with each other, which is quite different from the aquatic environment of inland lakes in the south and artificial reservoirs in the north in China (Chen et al., 2021). Before 2017, industrial wastewater, especially the wastewater of paper mill from Baoding City in Hebei province, was the main source of pollution, and human activities directly or indirectly affected the aquatic environment of Baiyangdian Lake (Zerizghi et al., 2020). Due to historical reasons, the water body and sediments in Baiyangdian Lake have been seriously polluted, causing water quality deterioration, high endogenous pollution loadings in sediments and severe polluted sediments deposition. Sediment is not only the storage place of pollutants in lakes and rivers, but also the source of pollutants themselves. In the previous study, with the increase in the discharge of antibiotics and other pollutants (such as heavy metals, persistent organic pollutants) into Baiyangdian Lake, the rapid and extensive growth of ARG has been accelerated (Cheng et al., 2014a, 2014b; Ji et al., 2019). Thus, most studies have demonstrated the occurrence of antibiotics in rivers or lakes, while the research regarding on the occurrence and relationship of antibiotics and ARGs simultaneously in different habitats of Baiyangdian Lake is relatively few. In this study, we describe the occurrence of antibiotics and ARGs in sediments of Baiyangdian Lake, aiming to track the effect of the different habitats including...
Fuhe river, reed marshes, open water, living areas, fish ponds and river estuary. It provides a more likely driving and influencing factor for the transmission of ARGs in lakes with complex and diverse habitats. Sediment samples were collected to determine correlations between ARGs, antibiotics, heavy metals and other environmental factors.

Materials and methods

Chemicals and standards

A total of 22 antibiotics widely detected in China were selected, there are 8 kinds of sulfonamides (SAs), 4 kinds of tetracyclines (TCs), 6 kinds of quinolones (QNs) and 4 kinds of macrodides (MLs). SAs include sulfamerazine (SMR), Sulfadimidine (SMZ), Sulfadiazine (SDZ), Sulfapyridine (SPD), Sulfamethoxypyridazine (SMP), Sulfaquinoxaline (SQX), Sulfamethoxazole (SMX), Sulfamethoxazole (STZ), QNs include Ciprofloxacin (CIP), Enrofloxacin (ENR), Lomefloxacin (LOM), Norfloxacin (NOR), Sparfloxacin (SPA), Ofloxacin (OFL), TCs include Tetracycline (TC), Doxycycline (DOC), Oxytetracycline (OTC), Chlortetracycline (CTC), MLs include Clarithromycin (CTM), Azithromycin (AZM), Dehydration Erythromycin (ERM-H2O) and Roxithromycin (RTM). All standards purchased from the laboratory of Dr. Ehrenstorfer (Augsburg, Germany). SMZ-d4 was used as an internal standard and purchased from ANPEL Laboratory Technologies (Shanghai, China). All chemicals or solvents are at least chromatographically pure, both the standard and the internal standard with a concentration of 1 mg/L are dissolved in methanol, and stored in amber glass vials at −20 °C for subsequent analysis.

Sampling site and sample collection

Baiyangdian Lake is located in the middle of Hebei province, with a surface area of 366 km² and an average depth of approximately 2–5 m (Yang et al., 2020). Surface sediments from six different habitats in Baiyangdian Lake were collected. From August to September 2020, 10 sediment samples in the Nanliuzhuang and Caiputai areas of Baiyangdian were determined (Fig. 1, Table S1). The characteristics of six habitats were as following: the Fuhe river (S1), the river estuaries (S2), the reed marshes (S3 and S9), the living area (S4), the fish ponds (S5-S8), and the open water (S10). Specifically, there are four different types of fishing ponds habitats: S5 is the former fishing ground that has been drained into the river, S6 is

Fig. 1 Distribution of sampling
the dredging, S7 is the successful in-situ bioremediation, and S8 is the failure of bioremediation (the algae used for bioremediation did not survive). Meanwhile, the reed marshes S3 is close to the river estuaries (S2), while S9 is in the center of large reed marshes area. The sediment was collected by a cylindrical sampling tube with a plexiglass tube (φ = 85 mm). After the sample was collected, the longitude and latitude coordinates, time and location information were recorded. Then 3 parallel samples around the same sampling point were taken, mixed evenly and placed in a glass bottle, and transported to the laboratory for processing within 24 h. Sections of the sediment were cut every 5 cm, and the surface sediment sample (0–5 cm) were collected for subsequent experiments.

Quantification of antibiotics

The sediment was pretreated using a multiphase solid phase extraction (SPE) system (supelco, USA) as previously described (Chen et al., 2014). In brief, 3 g of the sediment sample was accurately added in a 50 mL centrifuge tube, and the methanol/EDTA-Mcllvaine solution (1:1; V/V) mixed solvent was used as the extraction solution, then ultrasonically extract was performed twice. The extract was diluted to 500 mL with 0.1% EDTA aqueous solution, and its pH was acidified to 4.0 at the same time for later use. SPE was treated with oasis hydrophilic lipophilic balance (HLB) column (6 mL / 200 mg, waters, Milford, Ma, USA), then it was activated with 5 mL methanol and 5 mL water, respectively. The extract passed through HLB column at a flow rate of < 5 mL/min. The column was then washed with 3 mL 5% methanol and dried under vacuum for 0.5 h, followed by elution with 10 mL methanol. The extract was concentrated and nearly dried under nitrogen atmosphere. Then, the target extracts were redissolved in methanol water (1:1, V/V) solution.

The sediment extract samples were analyzed through liquid chromatography with tandem mass spectrometry (LC–MS/MS), which were determined according to the procedures described in previous study (Tong et al., 2020). The samples were analyzed by LC–MS/MS using an Accela Max 600 high performance liquid chromatography system with an online degasser, quaternary solvent transport system, automatic sampler, TSQ Quantum Access Max (ESI) source and electrospray ionization source. A Hypersil Goldtm C18 (100 mm × 2.1 mm, 1.9 μm, Thermofisher) was used at the flow rate of 0.3 mL/min and the oven temperature was set at 40 °C. The mobile phase was consisted of water containing 0.1% (V/V) formic acid (A) and methanol (B) with A gradual gradient distribution. Mass spectrometry was performed in positive ion mode, with sheath gas pressure of 40 bar, auxiliary gas pressure of 10 bar, carburetor temperature of 250 °C, capillary temperature of 350 °C and capillary offset of 35. Spray voltage is set to +3800 V. The analysis conditions for optimizing the MS/MS parameters (Du et al., 2017) are shown in Table S2. The recovery rate, limit of detection (LOD) and quantity (LOQ) were verified, and the results are shown in Table S3.

Quantification of ARGs

The sediment samples were sent to Shanghai Keyin Biological Technology (Shanghai, China) for analysis. Total DNA was extracted from 0.1 g fresh sediment samples by TIANNAMP Soil DNAKit (TIANGEN, China) following the manufacturer’s protocols. The DNA extracted from sediment samples was stored below −80 °C for further analysis. The quality and concentration of the resultant DNA were determined by Quawell Q3000 spectrophotometer (Quawell Technology, USA). Real-time quantitative fluorescence PCR was used to achieve quantitative and qualitative analysis of the starting template by real-time detection of the fluorescence signal corresponding to each cycle of PCR amplification products. In this experiment, SYBR fluorescent dye method was adopted. SYBR fluorescent dye was added into the PCR reaction system, and SYBR fluorescent dye was specifically mixed into the DNA double chain. The SYBR dye molecules that are not incorporated into the chain will not emit any fluorescence signal, thus ensuring that the increase in fluorescence signal is fully synchronized with the increase in PCR products.

The absolute abundance of two sul ARGs (suI1 and sul2), six tet ARGs (tetA, tetC, tetG, tetM, tetO and tetW), five qnr ARGs (qnrA, qnrC, qnrD, qnrS and aac (6′) -1B), four erm ARGs (ermA, ermB, ermC and ermF), three β-endamide ARGs (blaTEM-1, blaOXA-1 and ampC), 16S and intI1 was quantified by qPCR assays in sediment as previously described (Pu et al., 2018; Yang et al., 2018). The PCR product
were detected and analyzed using a StepOnePlus™ Realtime PCR (Thermo Fisher Scientific, Waltham, MA, USA). The primers for ARGs, correlation coefficients \( r^2 \) and standard curves of ARGs used in the study are shown in Table S4 and Table S5. qPCR reaction conditions were as follows: pre-denaturation at 95°C for 30 s, denaturation at 95 °C for 5 s, annealing extension at 60 °C for 30 s, repeated 40 cycles.

Environmental parameters in sediment

Total carbon (TC), total organic carbon (TOC), total nitrogen (TN), total phosphorus (TP), total sulfur (TS) and heavy metals (Zn, Cr, Ni, Cu, Pb, As, Cd and Hg) were determined in Baiyangdian Lake according to the Chinese standard geochemical sample analysis method (DZ/T 0279–2016).

Statistical analysis

Origin 2019 b and SPSS 26 software were used for statistical analysis of all data, linear regression was used for correlation analysis \( (P < 0.05 \) indicated significant differences), and redundancy analysis was conducted using the Canoco 5. Sampling sites were carried out by ArcMap10.6.

Results

Basic physicochemical properties

The nutrients (TC, TOC, TN, TP and TS) and heavy metals (Zn, Cr, Ni, Cu, Pb, As, Cd and Hg) of the sediments in different habitats are summarized in Table S6. 16S and intI1 was quantified by qPCR assays in sediment. From Fig. S1, it can be seen that TC, TOC, TN and TS are highest in the open water (S10) because a lot of decaying aquatic plants were found in the water nearby when we sampled. In addition to the open water, the content of the nutrients (TC, TOC and TN) in the river estuaries are much higher than other habitats, which indicated that the river estuaries were received the sewage from Fuhe River and nearby villages. Meanwhile, the lowest levels of the nutrients (TC, TOC, TP and TN) and heavy metals (Cu, Pb, Ni, As, Cd and Hg) were found in the fish pond, which was different from what we expected, possibly due to the fact that the fish ponds have been continuously repaired or treated recently. The mean contents of heavy metals in six habitats were followed as the order from highest to lowest, the Fuhe River, the river estuaries, the living area, the open water, the fish ponds and the reed marshes. Previous studies (Ji et al., 2019; Xu et al., 2011) have shown that the external heavy metal pollution in Baiyangdian Lake came from industrial pollution (e.g., lead storage battery plants, metal production plant, and chemical fiber factory). These factories were mainly located in Baoding district and their wastewater were imported into the upper reaches of Baiyangdian through Fuhe River. The high content of heavy metals in Fuhe River and the river estuaries was closely related to the industrial wastewater in Baoding City.

For 16S and intI1, 16S characterizes bacterial abundance and intI1 characterizes the level of gene migration (Hsu et al., 2014). As shown in Table.S8, the absolute abundance of 16S in different aquatic areas ranged from \( 1.25 \times 10^9 \) copies/g (S7) to \( 7.15 \times 10^8 \) copies/g (S3), with an average absolute content of \( 0.05 \times 10^9 \) copies/g. The absolute range of intI1 was \( 4.79 \times 10^6 \) copies/g (S2) to 0 copies/g (S5, S6, S9), with an average absolute abundance of \( 1.03 \times 10^6 \) copies/g. The higher abundance of 16S was found in open water, due to the decay of aquatic plants, which attract a large number of bacteria. And the lower abundance is concentrated in the reed marshes (S3), which was one tenth of that in the nearby estuary water (S2). The highest abundance of intI1 was found in the river estuarine, while the abundance of intI1 in the treated fish ponds and reed marshes was almost undetectable. It is possible that the reduction in pollutants after sediment treatment or under the action of reeds marshes inhibited the level of gene migration.

Occurrence of antibiotics in sediment

Table 1, Table S7 and Fig. 2 summarize the contents of all target antibiotic compounds in sediment samples from different waters area. More than 6 compounds tested were detected at 100% frequency in all samples in Baiyangdian Lake. The order of content of four kinds of antibiotics in different habitats was QNs, TCs, MLs, SAs. It can be seen that the presence of 6 different quinolones are common and seriously polluted antibiotics in sediments of different
habitats. CIP reached an especially high concentration of 104.94 ng/g in the river estuaries, and it was not detected in 8 of the remaining ten samples. Obviously, the maximum contents of QNs are seriously higher than the other antibiotics, especially SAs.

Among the six different habitats, the maximum contents of antibiotics were found in river estuary (S1), followed by the ecological restoration fish ponds (S7, S8), Fuhe river, open water, living area and reed marshes (S9). The antibiotic content of the sediment dredged treatment (S6) or the demolished fish ponds (S5) is significantly lower than that of the ecological restoration fish ponds (S7, S8), and the lowest antibiotic content was found in the reed marshes (S9).

Meanwhile, the antibiotic content in the reed marshes (S3) near the estuary was far lower than that in S2. Apparently, reeds have a significant removal effect on antibiotics.

Occurrence of AGRs

We detected 20 unique ARGs in all aquatic habitats, their relative abundance is shown in Fig. 3 and Table S8. Among all measured ARGs, the abundance

Table 1 Antibiotic contents in different habitats from the aquaculture environment of Baiyangdian Lake

| Compounds | Min. (μg/kg, dry/wt) | Max. | Mean | Fre/% |
|-----------|----------------------|------|------|-------|
| ERM-H₂O  | 0.04                 | 1.04 | 0.20 | 100.00|
| RTM      | 0.04                 | 2.41 | 0.32 | 100.00|
| CTM      | 0.02                 | 0.17 | 0.04 | 100.00|
| AZM      | <LOQ                 | 0.22 | 0.04 | 80.00 |
| TC       | 0.11                 | 2.25 | 0.25 | 100.00|
| DOC      | <LOQ                 | 2.14 | 0.20 | 90.00 |
| CTC      | <LOQ                 | 2.72 | 0.37 | 70.00 |
| OTC      | <LOQ                 | 31.03| 1.42 | 90.00 |
| SMR      | <LOQ                 | 0.10 | 0.01 | 70.00 |
| SPD      | <LOQ                 | 0.64 | 0.01 | 50.00 |
| SMP      | <LOQ                 | 0.05 | 0.01 | 30.00 |
| QNs      | <LOQ                 | 104.94| 22.62| – |
| MLs      | <LOQ                 | 2.41 | 1.00 | – |

Fig. 2 Contents of QNs, MLs, TCs and SAs

Fig. 3 Heat map of ARGs

*aLimits of quantification
of resistance genes was followed as the order, the sulfonamide resistance genes, quinolones resistance genes, macrolides, tetracycline resistance genes. Among the tested ARGs, sul1, sul2, aac(6’)-IB, qnrS, qnrD, blaTEM-1, ermF, tetG and tetM had 100% detection rate frequencies in all typical habitats. Sul1 was present in high relative abundance (6.21×10^7 copies/g) in all sediment samples, and the second was sul2 (4.48×10^7 copies/g), which were significantly higher than the other resistance genes. Furthermore, aac(6’)-IB (1.01×10^6–3.63×10^7 copies/g), as a qnr ARGs, the content was much higher than the other four qnr ARGs and the detection rate was found to be 100%. In addition to sul ARGs and qnr ARGs, both tet ARGs and β-endamid ARGs were frequently detected in all habitats. While except for ermF (5.13×10^3–4.40×10^5 copies/g, 100% detection rate), other three erm ARGs showed low detection frequencies (40–50%).

In terms of spatial distribution, the abundance of ARGs in different habitats of Nanliuzhuang were significantly higher than that of Caiputai. Meanwhile, the highest absolute abundance of ARGs were mainly concentrated near the Fuhe river (S1) and the river estuary (S2), and the lower value was mainly in fish ponds (S6-S8) and reed marshes (S9). In the case of fish ponds, the absolute abundance of the total ARGs in the fish ponds (S6) was 6.02×10^7 copies/g, which was much lower than that of the dredged and bioremediation ponds. Moreover, compared with two points (S3 and S9) in reed marshes, the total absolute abundance of ARGs in the sample point (S3) were significantly higher than the other (S9).

Impact of environmental factors on the absolute abundance of ARGs

Pearson correlation coefficients were calculated to examine the correlations between ARGs concentrations in different functional area of sediments (Table S9), the concentrations of TP and six ARGs had significant positive correlations (P<0.05). Heavy metals (Cu, Pb, Zn, Cr, Ni, Cd and Hg) and nine ARGs (ermB, ermF, sul1, sul2, tetA, tetG, tetM, blaOXA-1 and aac(6’)-IB) (p<0.05) exhibited significantly positive correlation. SAs and MLs appeared significant negatively correlated to sul1 (P<0.01), sul2 (P<0.01), and tetO (P<0.01), while QNs showed significant positive correlation with ermC (P<0.05), sul1 (P<0.05), and tetO (P<0.01). Furthermore, intI1 showed significant positive correlation with blaOXA-1 (P<0.05), ermC (P<0.01), sul1 (P<0.01), sul2 (P<0.01), tetM (P<0.05), tetO (P<0.01).

In this study, Redundancy analysis (RDA) was used to explain the relationship between basic physicochemical properties and ARGs in the six habitats. According to RDA (Fig. 4), ARGs are not only related to antibiotics, heavy metals and nutrients, but also related to each other, respectively. Redundancy analysis further confirmed the correlation analysis results among Cu, Zn, intI1, TP, MLs and ARGs.

Discussion

Presence of antibiotics in different habitats

Compared with SAs, MLs and TCs, QNs had the highest content in the sediments of different habitats in Baiyangdian Lake. This could result from their high viscosity leading to difficult to biodegrade, which promotes their persistence in the sediments (Moerno-Bondi et al., 2009). Meanwhile, the sorption coefficient of QNs was much higher than that of other antibiotics, which described the reversible adsorption and exchange of chemicals between water and sediment (Yang et al., 2010). The larger the sorption coefficient was, the stronger the chelating ability of the sediments was obtained with particles and cations (Li et al., 2012). Therefore, it is difficult to degrade and even accumulate in the sediments.

For all antibiotics tested, QNs were detected in all habitats in sediment samples, with the exception of certain low-frequency compounds such as CIP and NOR, which were almost absent in fish ponds and reed marshes. This may be due to its low mobility in fish ponds or reed marshes (Tong et al., 2020). The highest level of total antibiotics was found in the river estuary (S2) near the Fuhe river (S1), while the lowest level was found in reed marshes (S10), where water was constantly purified by the reeds around it. In the same way, the total amount of antibiotics in reed marshes (S3) near the estuary was significantly lower than that in the estuary river (S2). Besides, compared with the biological treatment fish ponds (S7 and S8), the total amount of antibiotics in the dredged (S6) or merged into rivers (S5) decreased significantly. These
results show that bioremediation may not effectively and quickly reduce the antibiotics in the surface sediments of fish ponds, due to the fact that aquatic plants mainly rely on root microorganisms to remove antibiotics in water (He et al., 2021), but in the short term, root microorganisms are inhibited by the high content of antibiotics in the sediments in the aquatic environment. And dredging or flowing into rivers can transfer antibiotics to achieve the purpose of less pollutants.

Diversity and abundance of ARGs

In this study, the ARGs were detected in different habitats of Baiyangdian Lake, which indicated that antibiotic resistance genes had been widespread in Baiyangdian Lake environment. Previous study (Chen et al., 2020) also indicated that abundant resistance genes were detected in the sediments of Baiyangdian Lake, leading to the high risk, and 80% ARGs were introduced from Fuhe River into Baiyangdian Lake. The risk of ARGs was higher than many lakes in Baiyangdian Lake, which was polluted by urban sewage, domestic sewage and aquaculture sewage (Chen et al., 2020; Zhang et al., 2020a). In this study, we also observed similar contents of ARGs in the Fuhe River and estuarine water. Apparently, the high detection rate and high absolute abundance of ARGs in estuarine water may be related to various pollution sources and human activities.

Sul ARGs are usually dominant among ARGs found in many sediments. Among all measured ARGs, the absolute abundance of sul1 was the highest, which is mobile DNA element-borne resistance gene, parallel transferring between bacterial populations and species (Hsu et al., 2014). The absolute abundance of sul2 is second only to sul1, which is similar to that of intI1, and its high abundance is closely related to intI1. For example, a significant correlation between sul ARGs and intI1 was found, and the contribution rate of sul ARGs was the highest in the environment in Japan (Partridge et al., 2009) and China (Suzuki et al., 2019). In addition to sul1 and sul2, the absolute abundance of aac(6′)-IB in qnr ARGs range was between 1.01 × 10^6 copies/g and 3.63 × 10^7 copies/g, and the detection rate was 100%. The distribution characteristics of this gene showed that the absolute abundance in the habitat near the Fuhe River and the estuaries river was one order of magnitude higher than that in other habitats, which might be related to the continuous input of industrial wastewater from the Fuhe River (Chen et al., 2020). Furthermore, although all the 13 erm ARGs, tet ARGs and β-endamidine ARGs were detected in varying degrees, most of them did not have high absolute
abundance. This may be related to their long persistence and not easy to disappear in the aquatic environment (Guo et al., 2018).

Factors influencing the ARG distribution

In the present study, ARGs in the main habitats positively correlated with the environmental factors. However, it was still difficult to explain the transmission of ARGs in different habitats of aquatic environments. Under the joint analysis of correlation analysis and RDA, the results showed that Cu, Zn, intI1, TP and MLs affected the absolute abundance of ARGs in different habitats of Baiyangdian Lake. This result is basically consistent with previous studies, which is showed that HMs promote the transfer of ARGs in the environment through the formation of reactive oxygen species, the increase in membrane permeability, the change of SOS response and the expression of conjugation related genes (Mckinney et al., 2010; Sun et al., 2021).

Particularly, it is widely accepted that when the concentration of antibiotics is higher than the background, the bacteria will become resistant bacteria through mutation or accelerated acquisition of exogenous resistance. At the same time, a large number of studies have shown that the abundance of surface resistance genes is positively correlated with antibiotics (Tong et al., 2020). Furthermore, in terms of the nutrients, TP is mainly related to ARGs. Previous studies found that the nutrients (TP) can affect microbial diversity and was one of the key factors of bacterial community structure change in Baiyangdian Lake (Leng et al., 2020; Zhang et al., 2020a). The influencing factors of ARGs in the mono-habitat lakes are still related to antibiotics, HMs, nutrients and integrons, while the mono-habitat lakes are often affected by heavy metal or antibiotic pressure (Tong et al., 2020; Zhao et al., 2020; Zhou et al., 2019). Therefore, due to the complex habitat of Baiyangdian Lake and the human activities result in the input of ARGs into lakes through Fuhe River, these results indicated that there are some differences in the influencing factors of resistance genes between the multi-habitat lakes and mono-habitat lakes. On the whole, it is proved that these environmental factors play a certain role in the spread of ARGs. Therefore, it is necessary to discuss the propagation mechanism and risk assessment method of ARGs.

Conclusions

In summary, the study represented the occurrence of antibiotics and ARGs in Fuhe river, the estuaries river, the reed marshes, the living area, the fish ponds, and the open water of Baiyangdian Lake. QNs were dominant among the antibiotics, especially in the river entrance habitat, and the highest content is up to 104.94 ng/g. The 20 kinds of ARGs, intI1 and 16S have been detected with varying degrees in different habitats, indicating that Baiyangdian Lake is an important reservoir of ARGs. Thereinto, sul1 had the highest absolute abundance \((6.21 \times 10^7 \text{ copies/g},)\) which was mobile DNA element-borne resistance gene. Furthermore, redundant analysis showed that Cu, Zn, intI1, TP and MLs were important factors affecting the abundance and distribution of ARGs in the sediments of different habitats of Baiyangdian Lake. In addition, the content of antibiotics in water, plants and animals in the aquatic environment also affects the production of ARGs. The correlation between bacteria and ARGs cannot be ignored and may interact to promote the spread and generation of ARGs. Given this situation, it is necessary to further study the synergistic effect of antibiotics and heavy metals on the source and fate of resistance genes, as well as the potential harm to human beings.

**Author contributions** All authors contributed to the study conception and design. GL gave the idea of this research work. TW Material preparation, data collection and analysis were performed. The first draft of the manuscript was written by GL and TW. MZ, LL, WZ and DW helped the work with his ideas and made a critical editing and reviewing of the whole manuscript. All authors read and approved the final manuscript.

**Funding** This work was supported by the National Natural Science Foundation of China (No. 52030003) and the Major Science and Technology Program for Water Pollution Control and Treatment (2018ZX07110004).

**Data availability** Not applicable.

**Declarations**

**Conflict of interest** The authors declare no competing interests.

**Consent to participate** Not applicable.

**Consent for publication** Not applicable.

**Ethical approval** Not applicable.
References

Amarasiri, M., Sano, D., & Suzuki, S. (2020). Understanding human health risks caused by antibiotic resistant bacteria and antibiotic resistance genes in water environments: Current knowledge and questions to be answered. Critical Reviews in Environmental Science and Technology, 50(19), 2016–2059.

Bai, Y., Ruan, X., Li, R., Zhang, Y., & Wang, Z. (2021). Metagenomics-based antibiotic resistance genes diversity and prevalence risk revealed by pathogenic bacterial host in taihu lake, China. Environmental Geochemistry and Health, 44(8), 2531–2543.

Chen, H., Jing, L., Teng, Y., & Wang, J. (2018). Characterization of antibiotics in a large-scale river system of china: Occurrence pattern, spatiotemporal distribution and environmental risks. Science of the Total Environment, 618, 409–418.

Chen, H., Jing, L., Yao, Z., Meng, F., & Teng, Y. (2019a). Prevalence, source and risk of antibiotic resistance genes in the sediments of lake tai (China) deciphered by metagenomic assembly: A comparison with other global lakes. Environment International, 127, 267–275.

Chen, H., Li, Y., Sun, W., Song, L., & Teng, Y. (2020). Characterization and source identification of antibiotic resistance genes in the sediments of an interconnected river-lake system. Environment International, 137, 105538.

Chen, J., Su, Z., Dai, T., Huang, B., Mu, Q., Zhang, Y., et al. (2019b). Occurrence and distribution of antibiotic resistance genes in the sediments of the east China sea bays. Journal of Environmental Sciences (China), 81, 156–167.

Chen, J., Wu, J., Liu, M., Li, L., Zhang, W., Wang, D., et al. (2021). Bacterial community structure in the surface sediments of different habitats of baiyangdian lake, northern china: Effects of nutrient conditions. Journal of Soils and Sediments, 21(4), 1866–1874.

Chen, K., & Zhou, J. L. (2014). Occurrence and behavior of antibiotics in water and sediments from the Huangpu river, Shanghai, China. Chemosphere, 95, 604–612.

Cheng, D., Liu, X., Liang, W., Gong, W., Liu, G., Fu, W., et al. (2014a). Seasonal variation and sediment–water exchange of antibiotics in a shallower lake in north china. Science of the Total Environment, 476–477, 266–275.

Cheng, M., Wu, L., Huang, Y., Luo, Y., & Christie, P. (2014b). Total concentrations of heavy metals and occurrence of antibiotics in sewage sludges from cities throughout china. Journal of Soils and Sediments, 14(6), 1123–1135.

Czekalski, N., Sigdel, R., Birtel, J., Matthews, B., & Buergmann, H. (2015). Does human activity impact the natural antibiotic resistance background? abundance of antibiotic resistance genes in 21 swiss lakes. Environment International, 81, 45–55.

Dong, Z. K., Wang, J. H., Wang, L. J., Zhu L. S., Wang, J., Zhao, X., & Kim, Y. M. (2021). Distribution of quinolone and macrolide resistance genes and their co-occurrence with heavy metal resistance genes in vegetable soils with long-term application of manure. Environmental Geochemistry and Health, pp. 1–16.

Du, J., Zhao, H., Liu, S., Xie, H., Wang, Y., & Chen, J. (2017). Antibiotics in the coastal water of the South Yellow sea in China: Occurrence, distribution and ecological risks. Science of the Total Environment, 595, 521–527.

Gillings, M. R., Gaze, W. H., Pruden, A., Smalla, K., Tiedje, J. M., & Zhu, Y. G. (2014). Using the class 1 integron-integrase gene as a proxy for anthropogenic pollution. Isme Journal, 9(6), 1269–1279.

Gulkowska, A., Leung, H. W., So, M. K., Taniyasu, S., Yamashita, N., Yeung, L., et al. (2008). Removal of antibiotics from wastewater by sewage treatment facilities in hong kong and Shenzhen, China. Water Research, 42, 395–403.

Guo, X. P., Liu, X., Niu, Z. S., Lu, D. P., Zhao, S., Sun, X. L., et al. (2018). Seasonal and spatial distribution of antibiotic resistance genes in the sediments along the Yangtze estuary, China. Environmental Pollution, 242, 576–584.

Han, X. M., Hu, H. W., Shi, X. Z., Wang, J. T., Han, L. L., Chen, D. L., & He, J. Z. (2016). Impacts of reclaimed water irrigation on soil antibiotic resistome in urban parks of Victoria, Australia. Environmental Pollution, 211, 48–57.

He, Y. J., Zhou, K. P., Rao, Y. X., & Ji, R. (2021). Environmental risks of antibiotics in soil and the related bioremediation technologies. Chinese Journal of Biotechnology., 37(10), 3487–3504.

Hsu, J. T., Chen, C. Y., Young, C. W., Chao, W. L., Li, M. H., Liu, Y. H., Lin, C. M., & Ying, C. W. (2014). Prevalence of sulfonamide-resistant bacteria, resistance genes and integron-associated horizontal gene transfer in natural water bodies and soils adjacent to a swine feedlot in northern Taiwan. Journal of Hazardous Materials, 277, 34–43.

Ji, Z. H., Zhang, H., Zhang, Y., Chen, T., Long, Z. W., Li, M., & Pei, Y. S. (2019). Distribution, ecological risk and source identification of heavy metals in sediments from the Baiyangdian Lake, Northern China. Chemosphere, 237, 124425.

Kim, J. H., Kuppusamy, S., Kim, S. Y., Kim, S. C., Kim, H. T., & Lee, Y. B. (2017). Occurrence of sulfonamide class of antibiotics resistance in Korean paddy soils under long-term fertilization practices. Journal of Soils and Sediments, 17, 1618–1625.

Klein, E. Y., VanBoeckel, T. P., Martinez, E. M., Pant, S., Gandara, S., Levin, S. A., Goossens, H., & Laxminarayan, R. (2018). Global increase and geographic convergence in antibiotic consumption between 2000 and 2015. PNAS, 115, 3463–3470.

Knapp, C. W., Dolfing, J., Ehlert, P. A. I., & Graham, D. W. (2010). Evidence of increasing antibiotic resistance gene abundances in archived soils since 1940. Environmental Science & Technology, 44, 580–587.

Kummerer, K. (2009). Antibiotics in the aquatic environment—A review-part I. Chemosphere, 75, 417–434.

Larson, E. (2007). Community factors in the development of antibiotic resistance. Annual Review of Public Health, 28, 435–447.

Leng, Y. F., Xiao, H. L., Li, Z., & Wang, J. (2020). Tetracyclines, sulfonamides and quinolones and their corresponding resistance genes in coastal areas of Beibu Gulf, China. Science of The Total Environment, 714, 1368991–1368999.

Li, A. L., Chen, L. J., Zhan, Y., Tao, Y. L., Xie, H., Li, S., Sun, W. L., Pan, J. G., He, Z. D., Mai, C. A., Fan, Y. Y., Xian.
H. C., Zhang, Z. B., & Wen, D. H. (2018a). Occurrence and distribution of antibiotic resistance genes in the sediments of drinking water sources, urban rivers, and coastal areas in Zhuhai, China. *Environmental Science & Pollution Research*, 25(26), 26209.

Li, J., Cao, J. J., Zhu, Y. G., Chen, Q. L., Shen, F. X., Wu, Y., Xu, S. Y., Fan, H., Da, G., Huang, R. J., Wang, J., DeJesus, A. L., Morawska, L., Chan, C. K., Peccia, J., & Yao, M. S. (2018b). Global survey of antibiotic resistance genes in air. *Environmental Science & Technology*, 52, 10975–10984.

Li, W. H., Shi, Y. L., Gao, L. H., Liu, J. M., & Cai, Y. Q. (2012). Occurrence of antibiotics in water, sediments, aquatic plants, and animals from Baiyangdian Lake in North China. *Chemosphere*, 89, 1307–1315.

Liu, X. H., Zhang, G. D., Liu, Y., Lu, S. Y., Qin, P., Guo, X. C., Bi, B., Wang, L., Xi, B. D., Wu, F. C., Wang, W. L., & Zhang, T. T. (2019). Occurrence and fate of antibiotics and antibiotic resistance genes in typical urban water of Beijing, China. *Environmental Pollution*, 246, 163–173.

Luo, L., Ashworth, D., Dungan, R. S., Xuan, R., & Yates, S. R. (2010). Trends in antibiotic resistance genes occurrence in the Haihe River, China. *Environmental Science & Technology*, 44, 7220–7225.

Mccann, C. M., Christgen, B., Roberts, J. A., Su, J. Q., Arnold, K. E., Gray, N., et al. (2019). Understanding drivers of antibiotic resistance genes in high arctic soil ecosystems. *Environmental International*, 125, 497–504.

Mckinney, C. W., Loftin, K. A., Meyer, M. T., Davis, J. G., & Pruden, A. (2010). Tet and sul antibiotic resistance genes in livestock lagoons of various operation type, configuration, and antibiotic occurrence. *Environmental Science & Technology*, 44(16), 6102–6109.

Moreno-Bondi, M. C., Marazuela, M. D., Herranz, S., & Rodriguez, E. (2009). An overview of sample preparation procedures for lc-ms multiclass antibiotic determination in environmental and food samples. *Analytical & Bioanalytical Chemistry*, 395(4), 921–946.

Muhammad, J., Khan, S., Su, J. Q., Hesham, A-E-L., Ditta, A., Nawab, J., & Ali, A. (2020). Antibiotics in poultry manure and their associated health issues: A systematic review. *Journal of Soils and Sediments*, 20, 486–497.

Nguyen, T. N., Kasuga, I., Liu, M., & Katayama, H. (2019). Occurrence of antibiotic resistance genes as emerging contaminants in watersheds of tama river and lake kasumigaura in japan. *IOP Conference Series Earth and Environmental Science*, 266, 12003–12012.

Partridge, S. R., Guy, T., Enrico, C., & Fredell, J. R. (2009). Gene cassettes and cassette arrays in mobile resistance integrons. *Fems Microbiology Reviews*, 33(4), 757–784.

Paulus, G. K., Hornstra, L. M., & Medema, G. (2020). International tempo-spatial study of antibiotic resistance genes across the Rhine river using newly developed multiplex qPCR assays. *Science of the Total Environment*, 706, 135733.

Pruden, A., Arabi, M., & Storteboom, H. N. (2012). Correlation between upstream human activities and riverine antibiotic resistance genes. *Environmental Science & Technology*, 46, 11541–11549.

Pu, C. J., Liu, H., Ding, G. C., Sun, Y., Yu, X. L., Chen, J. H., Ren, J. Y., & Gong, X. Y. (2018). Impact of direct application of biogas slurry and residue in fields: In situ analysis of antibiotic resistance genes from pig manure to fields. *Journal of Hazardous Materials*, 344, 441–449.

Quai, S., Embrandiri, A., Ravindran, B., Hossain, K., & Ignacimuthu, S. (2019). Veterinary antibiotics in animal manure and manure laden soil: Scenario and challenges in Asian countries. *Journal of King Saud University-Science*, 32(2), 1300–1305.

Reichert, G., Hilgert, S., Alexander, J., Azevedo, J., & Schwartz, T. (2021). Determination of antibiotic resistance genes in a wwt-impacted river in surface water, sediment, and biofilm: Influence of seasonality and water quality. *Science of the Total Environment*, 768, 144526.

Sun, F. L., Xu, Z. T., & Fan, L. L. (2021). Response of heavy metal and antibiotic resistance genes and related microorganisms to different heavy metals in activated sludge. *Journal of Environmental Management*, 300, 113754.

Suzuki, S., Nakamichi, S., Tamminen, M., Yokokawa, T., Sato-Takabe, Y., Ohta, K., Chou, H. Y., Muziasari, W. I., & Virta, M. (2019). Occurrence of sul and tet(m) genes in bacterial community in Japanese marine aquaculture environment throughout the year: Profile compari-son with taiwanese and finnish aquaculture waters. *Science of the Total Environment*, 669(15), 649–656.

Tong, L., Qin, L. T., Guan, C., Wilson, M. E., Li, X. J., Cheng, D. D., Ma, J., Liu, H., & Gong, F. J. (2020). Antibiotic resistance gene profiling in response to antibiotic usage and environmental factors in the surface water and groundwater of Honghu Lake, China. *Environmental Science & Pollution Research International*, 27, 31995–32005.

Xu, F., Yang, Z. F., Chen, B., & Zhao, Y. W. (2011). Ecosystem health assessment of the plant-dominated Baiyangdian lake based on eco-exergy. *Ecological Modelling*, 222(1), 201–209.

Xu, Y., Guo, C. S., Luo, Y., Lv, J. P., Zhang, Y., Lin, H. X., Wang, L., & Xu, J. (2016). Occurrence and distribution of antibiotics, antibiotic resistance genes in the urban rivers in Beijing, China. *Environmental Pollution*, 213, 833–840.

Yang, J. F., Ying, G. G., Zhao, J. L., Tao, R., Su, H. C., & Guo, C. S., Luo, Y., Lv, J. P., Zhang, Y., Lin, H. X., Wang, L., & Xu, J. (2016). Occurrence and distribution of antibiotics, antibiotic resistance genes in the urban rivers in Beijing, China. *Environmental Pollution*, 213, 833–840.

Yang, J. F., Ying, G. G., Zhao, J. L., Tao, R., Su, H. C., & Chen, F. (2010). Simultaneous determination of four classes of antibiotics in sediments of the pearl rivers using rrlc-ms/ms. *Science of the Total Environment*, 408(16), 3424–3432.

Yang, W., Yan, J., Wang, Y., Zhang, B. T., & Wang, H. J. (2020). Seasonal variation of aquatic macrophytes and its relationship with environmental factors in Baiyangdian Lake, China. *Science of the Total Environment*, 708, 135112.

Yang, X. L., Zhang, S., Li, H., Zhang, L. M., Song, H. L., & Wang, Y. W. (2018). Effects of voltage on sulfadiazine degradation and the response of sul genes and microbial communities in biofilm-electrode reactors. *Ecotoxicology and Environmental Safety*, 151, 272–278.

Yang, Y., Liu, W., Xu, C., Wei, B., & Wang, J. (2017). Antibiotic resistance genes in lakes from middle and lower reaches of the Yangtze river, China: Effect of land use and sediment characteristics. *Chemosphere*, 178, 19–25.

Zerizghi, T., Yang, Y., Wang, W., Zhou, Y., & Yi, Y. (2020). Ecological risk assessment of heavy metal concentrations in sediment and fish of a shallow lake: A case study of
baiyangdian lake, north china. *Environmental Monitoring and Assessment, 192*(2), 154.1-154.16.

Zhang, L., Shen, L., Ju, Z., Fu, Y., Qin, S., & Cui, J. (2020a). The key environmental influencing factors for the change of sediment bacterial community and antibiotics resistance genes in a long-term polluted lake, china. *Ecotoxicology, 30*, 1538–1549.

Zhang, Q. Q., Ying, G. G., Pan, C. G., Liu, Y. S., & Zhao, J. L. (2015). Comprehensive evaluation of antibiotics emission and fate in the river basins of china: Source analysis, multimedia modeling, and linkage to bacterial resistance. *Environmental Science & Technology, 49*(11), 6772–6782.

Zhang, T. Q., Lu, K. Y., Lu, Q. X., Wang, L., & Liu, X. W. (2021). Removal of antibiotic-resistant genes during drinking water treatment: A review. *Journal of Environmental Sciences (China), 104*, 415–429.

Zhang, Y., Chen, H., Jing, L., & Teng, Y. (2020b). Ecotoxicological risk assessment and source apportionment of antibiotics in the waters and sediments of a peri-urban river. *Science of the Total Environment, 731*, 139128.

Zhao, B., Xu, J., Zhang, G., Lu, S., & Li, M. (2020). Occurrence of antibiotics and antibiotic resistance genes in the fuxian lake and antibiotic source analysis based on principal component analysis-multiple linear regression model. *Chemosphere, 262*, 127741.

Zhao, J. L., Liu, Y. S., Liu, W. R., Jiang, Y. X., Su, H. C., Zhang, Q. Q., Chen, X. W., Yang, Y. Y., Chen, J., Liu, S. S., Pan, C. G., Huang, G. Y., & Ying, G. G. (2015). Tissue-specific bioaccumulation of human and veterinary antibiotics in bile, plasma, liver and muscle tissues of wild fish from a highly urbanized region. *Environmental Pollution, 198*, 15–24.

Zheng, J., Zhou, Z., Wei, Y., Chen, T., & Chen, H. (2018). High-throughput profiling of seasonal variations of antibiotic resistance gene transport in a peri-urban river. *Environmental International, 114*, 87–94.

Zhou, Q., Wang, M. Z., Zhong, X. X., Liu, P., Xie, X. Y., Wangxiao, J. Y., & Sun, Y. X. (2019). Dissemination of resistance genes in duck/fish polyculture ponds in guangdong province: Correlations between cu and zn and antibiotic resistance genes. *Environmental Science & Pollution Research International, 26*, 8182–8193.

Zhu, Y. G., Johnson, T. A., Su, J. Q., Qiao, M., Guo, G. X., Stedtfeld, R. D., et al. (2013). Diverse and abundant antibiotic resistance genes in chinese swine farms. *PNAS, 110*(9), 3435–3440.

Publisher’s Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.