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Metagenomic insights into the profile of antibiotic resistomes in a large drinking water reservoir

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ABSTRACT

Reservoirs play a vital role in the control and management of surface water resources. However, the long water residence time in the reservoir potentially increases the storage and accumulation of antibiotic resistant genes (ARGs). The full profiles and potential health risks of antibiotic resistomes in reservoirs are largely unknown. In this study, we investigated the antibiotic resistomes of water and sediment during different seasons in the Danjiangkou Reservoir, which is one of the largest reservoirs in China, using a metagenomic sequencing approach. A total of 436 ARG subtypes belonging to 20 ARG types were detected from 24 water and 18 sediment samples, with an average abundance of 0.138 copies/cell. The overall ARG abundance in the sediment was higher than that in the water, and bacitracin and vancomycin resistance genes were the predominant ARG types in the water and sediment, respectively. The overall ARG abundance in the dry season was higher than that in the wet season, and a significant difference in ARG subtype compositions was observed in water, but not in the sediment, between the different seasons. The potential horizontal gene transfer frequency in the water was higher than that in the sediment, and the ARGs in water mainly came from the sediment upstream of the reservoir. The metagenomic assembly identified 14 contigs as ARG-carrying pathogens including *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*, and 3 of 14 carried virulence factors. Overall, the potential public health risks posed by resistomes in the water of the Danjiangkou Reservoir were higher in the dry season than in the wet season. Based on these results, strategies including sediment control and pathogen monitoring are suggested for water safety management in drinking water reservoirs.

1. Introduction

The extensive use of antibiotics has led to the widespread emergence of antibiotic resistant bacteria (ARB), and antimicrobial resistance is becoming an emerging global challenge to public health (Berendonk et al., 2015; Christou et al., 2017). The antibiotic resistant genes (ARGs) encoding resistance to antibiotics have become an emerging environmental pollutant that need more attention (Li et al., 2015b; Pruden et al., 2006). ARGs have been widely detected in various environments, such as hospitals (Rodriguez-Mozaz et al., 2015), wastewater treatment plants (Che et al., 2019; Su et al., 2017), soil (Forsberg et al., 2014; Su et al., 2014), rivers (Zhang et al., 2015; Zhu et al., 2017b) and drinking water (Ma et al., 2017). These environmental ARGs can transfer among bacteria through horizontal gene transfer (HGT) via conjugation, transduction or transformation (Mao et al., 2014; Wang et al., 2019). Once pathogenic bacteria uptake the ARGs and gain resistance to antibiotics, they become a serious threat to human health (Jiang et al., 2017; Zhu et al., 2017a). Therefore, exploring the distribution and dissemination patterns of environmental ARGs is essential for controlling the ARG pollution and for reducing the risk of pathogens to human health.

Surface water is the main source of drinking water for residents, and its quality is directly related to human health (Bai et al., 2019). However, previous studies have reported that surface water harbors a wide variety of ARGs and has become the “reservoir” of ARGs (Shao et al., 2018). Moreover, approximately 16.7 million reservoirs larger than 0.01 ha have been built around the world, with a combined storage capacity of approximately 8070 km^3 of surface water (Lehner et al.,

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2011). Due to the long residence time of water in the reservoir, it is possible for reservoirs to store and accumulate ARGs to a greater extent than rivers (Czekalski et al., 2015; Liu et al., 2018b), which highlights the importance of research on the resistome in reservoirs. Previous studies mainly used conventional methods, such as isolation and quantitative polymerase chain reaction (qPCR) to search for ARGs in reservoirs (Chen et al., 2019; Czekalski et al., 2015; Yan et al., 2018), and none of this exploratory work had simultaneously investigated the ARGs accumulation and dissemination pattern between the water body and sediment. Most importantly, the potential health risks posed by ARGs in reservoirs are still largely unknown, as the horizontal transfer frequency, sources and hosts of ARGs have not been thoroughly studied.

The Danjiangkou Reservoir is located on the Han River (the longest tributary of the Yangtze River in China), and it is the place where the Dan River flows into. Water in the Danjiangkou Reservoir is mainly from the Han River and Dan River, and the total area is approximately 1023 km^2, with a water storage capacity of 29 billion m^3 (Li et al., 2019). Moreover, the Danjiangkou Reservoir is the water source for the middle route of the South-to-North Water Diversion Project, which is the largest water transfer project in the world. As of December 2018, the Danjiangkou Reservoir had provided 17.9 billion m^3 of water to 53.0 million people in Beijing, Tianjin, Hebei and Henan provinces over four years (http://nsbd.mwr.gov.cn/). Thus, the Danjiangkou Reservoir can be chosen as a representative of the large drinking water reservoir system to explore the profile of ARGs and their potential risks to public health. A recent study showed that a wide spectrum of antibiotics had been detected in the Danjiangkou Reservoir at a concentration of 149 ng/L in water and 12.1 ng/g in sediment (Li et al., 2019), which might induce the accumulation of ARB within the reservoir, highlighting the importance of a timely systematic investigation of antimicrobial resistance within this important drinking water source of north China.

In this study, we investigated the ARGs of water and sediment in the Danjiangkou Reservoir in different seasons using a metagenomic sequencing approach, with the aim of (1) providing a blueprint of seasonal variation in the ARG profile in the water and sediment of the Danjiangkou Reservoir; (2) estimating the horizontal transfer frequency and source of ARGs in the reservoir; (3) identifying the ARG hosts, especially the ARG-carrying pathogens; and (4) proposing accessible reservoir management strategies to control the ARG contamination.

2. Methods and materials

2.1. Sample collection

The Danjiangkou Reservoir (N32°20′-33°10′, E111°00′-111°50′) is located on the Han River (the longest tributary of Yangtze River) and consists of the Han River reservoir and the Dan River reservoir. The sampling campaigns were carried out in April (the dry season) and October (the wet season) in 2016 to investigate the seasonal characteristics. In each sampling campaign, water and sediment samples were collected from 12 sites in the Danjiangkou Reservoir, including 5 sites (DSQ, BDT, DKKX, TC and LSH) in the Dan River reservoir area (no sediment sample was collected at site TC), 5 sites (XC, LK, HK, LH and LHKX) in the Han River reservoir area, one site (DJKBS) upstream of the Danjiangkou dam, and one site (DJKBX) downstream of the Danjiangkou dam (Fig. S1). Totally, 46 samples (24 water samples and 22 sediment samples) were collected. The "DJKBX" site was located downstream of the dam and was not in the scope of the water source of the South-to-North Water Diversion Project. In addition, sewage water from surrounding cities also flows into this site.

The 20 L mixed water samples were collected across three depths: surface (0.5 m), middle (20–30 m) and bottom (40–50 m) water layers at each site, stored in sterile polyethylene terephthalate bottles, and immediately transported to the laboratory on ice. The 20 L water sample was split into two parts: 5 L was stored at 4 °C for physicochemical properties analysis; the remaining 15 L water was filtered through 0.22-μm membranes (Millipore, USA) to capture microbial cells, and the filtered membranes were stored at −80 °C for DNA extraction. Surface sediment samples were collected where flow depth was about 0.5 m and sealed in 50-mL sterilized polypropylene tubes and immediately transported to the laboratory in dry ice. The sediment sample from each site was split into two parts: one part was stored at −80 °C for DNA extraction; the other part was stored at 4 °C for physicochemical properties analysis. The methods for physicochemical properties analyses on water and sediment samples were described previously (Dang et al., 2018). The physicochemical properties were listed in Table S1.

2.2. DNA extraction and sequencing

Three biological duplication of filtered membranes and frozen sediment samples were used to extract DNA using the FastDNA® SPIN Kit for Soil (MP Biomedicals, USA) according to manufacturer's protocols. DNA concentrations and purity were determined by NanoDrop One instrument (Thermo Fisher Scientific, Wilmington, DE, USA). The duplicate DNA extracts were finally mixed for the following sequencing step. The V3-V4 hypervariable regions of 16S rRNA genes were amplified by PCR using the barcoded primer set 338F (5′-ACTCCTACGG GAGGCAGCA-3′) and 806R (5′-GGACTACCAGGGTATCTAAT-3′) (Mori et al., 2014). Amplicons were extracted from 2% agarose gels and purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA) according to the manufacturer's instructions. Purified amplicons of 16S rRNA genes were sequenced using Illumina MiSeq platform (Majorbio Company in Shanghai) with the strategies of PE250 (paired-end sequencing 250 × 2). No-template samples were performed as negative controls to monitor any contamination during all the workflow process. The raw sequences were assigned to each samples according to unique barcode, and the sequences with average quality scores > 30 (Q30) and length > 400 bp were reserved. These sequences were denoised and generated sub-operational taxonomic units (sOTUs) using the Deblur plugin in QIIME2 pipeline with the default parameters (https://docs.qiime2.org). To compare the sOTUs abundance between different samples, the sequencing depth of each sample was normalized to 11,764 reads. The taxonomy analyses of sOTUs were conducted using feature-classifier plugin in QIIME2 with the Greengenes database (v. 13_8) at 99% similarity (DeSantis et al., 2006).

The shot gun metagenomic sequencing of DNA extracted from water and sediment samples were performed simultaneously. Briefly, approximately 1 μg DNA was used to construct library with a 300 bp insert size, followed by sequencing using Illumina HiSeq 4000 platform (Majorbio Company in Shanghai) with the strategies of PE150 (paired-end sequencing 150 × 2). 4 samples failed to construct library due to their low DNA concentrations, and finally 42 samples were subjected to metagenomic sequencing. The raw sequences were assigned to each sample according to unique barcode, and the adapter was cut using Seqprep (https://github.com/jstjohn/SeqPrep). After that, the sequences with average quality scores < 30 (Q30) or length < 50 bp were removed using Sickle (https://github.com/najoshi/sickle). The detailed information about the datasets of these samples were summarized in Table S2. The data were deposited into the NCBI Sequence Read Archive (SRA) database under accession number PRJNA558512.

2.3. Calculations of ARG abundance

The ARGs were identified and calculated using ARGs-OAP v2.0 based on the clean short reads (Yin et al., 2018). To compare the ARG abundance in different samples, we normalized the ARG abundance against the cell numbers in each sample. The relative ARG abundance was finally represented as ARG copies per cell (copies/cell). Briefly, the ARG reads were identified against the SARG database at the cutoff of

10^{-7} E-value, 80% identity and 75% hit length. The reads were used to search the database of 30 essential single-copy gene (ESCG) families, which are found in nearly all bacteria and archaea and are bacterial and archaeal PhyEco marker genes (Nayfach and Pollard, 2015). The average coverage of ESCGs was used to calculate the cell numbers in each sample.

2.4. Metagenome assembly and gene prediction

After quality control, the clean reads were assembled using IDBA-UD (Peng et al., 2012), and the assembled contigs longer than 500 bp were reserved. We obtained 12567853 contigs with an average length of 1221.9 bp from 42 metagenomic samples. The open reading frames (ORFs) of the contigs were predicted using Prodigal v2.6.3 with a meta model (Hyatt et al., 2010). The coverage of each contig was calculated by mapping clean reads to the contigs using bbmap (https://sourceforge.net/projects/bbmap/) with the default parameters.

2.5. Identification of ARG-like ORFs and bacterial hosts of ARGs

The ARG-like ORFs were identified against the SARG database using DIAMOND (Buchfink et al., 2015) with an E-value $\leq 10^{-10}$, and the results that identity $\geq$ 80% and hit length $\geq$ 70% its length were identified as ARG-like ORFs (Ma et al., 2016). To compare coverage between different samples, the ARG-like ORFs coverage was normalized by the data size of each sample (copies/Gb). The calculation formula was as follows:

$$\text{Coverage} = \sum_{1}^{n} \frac{N \times 150/L}{G}$$

where n means the total number of ARG-like ORFs in one sample, N means the number of clean reads mapped to ARG-like ORFs, 150 is the length of clean reads, L means the length of the target ARG-like ORFs, and the G means the data size (Gb) of clean reads per sample (Ma et al., 2016; Xiong et al., 2018).

The ORFs sequences on the contigs that carried ARG-like ORFs were searched against the NCBI RefSeq database using BLASTP with an E-value $\leq 10^{-5}$. The results were parsed using MEGAN (version 5) (Huson et al., 2016) and the contigs were annotated as the taxon if more than 50% of ORFs on the contig were annotated as the same taxon (Ishii et al., 2013).

The ARG-carrying contigs at species level were then searched against the previous summarized pathogen list to identify ARG-carrying pathogens (Li et al., 2015a). The virulence factors of ARG-carrying pathogen were detected by using VFanalyzer on VFDB (Virulence Factors of Pathogenic Bacteria) (Liu et al., 2019).

2.6. Statistical analysis

The principal co-ordinates analysis (PCoA) was performed to describe the similarity of community at the sOTUs level and ARG subtypes using the ggord package in R3.5.2 (https://www.r-project.org/). Analysis of similarity (ANOSIM) statistics was calculated to test the significance of differences in bacterial community and ARG types using the vegan package in R3.5.2.

Network analysis was used to explore the potential correlations between bacteria and ARGs. The Spearman's correlation coefficients (ρ) were calculated using the vegan package in R3.5.2. The sOTUs or ARGs subtypes that co-occurred in at least 50% samples were reserved to calculate Spearman's correlation coefficients. The correlation between two nodes was considered as statistically significant when $\rho \geq 0.8$ and P value ≤ 0.01. The network analysis was performed using igraph package in R3.5.2 and then visualized in Gephi (version 0.9.9).

2.7. HGT analysis

To estimate the potential HGT of ARGs in water and sediment samples, Procrustes analysis based on the Bray-Curtis distances of sOTUs and ARG subtypes were performed using QIIME (Caporaso et al., 2010) following the previous steps (Forsberg et al., 2014). The M^2 value (the sum of squared distances between matched-sample PCoA plots) and P value were computed by 10,000 label permutations. Based on the hypothesis that a significant correlation between sOTUs and ARG subtypes was observed, this indicated that the bacterial community structure determined the distribution of the resistome and the HGT of ARGs was not sufficient to obscure their association with bacterial genomes.

Additionally, the ORF annotation results were used to identify the mobile genetic elements (MGEs) based on string matches to one of the following keywords: transposase, transposon, conjugative, integrase, integron, recombinase, conjugal, mobilization, recombination and plasmid (Forsberg et al., 2014, Ju et al., 2019). To compare the MGE abundance among different samples, the MGE coverage was also normalized by the data size of each sample (copies/Gb) according to the above formula.

2.8. Source tracking analysis

SourceTracker uses a Bayesian classification approach with Gibbs sampling to estimate the proportion of marker-genes in a given set of sink environments that come from possible source environments (Knights et al., 2011; Thompson et al., 2017). We applied SourceTracker (http://sourcetracker.sf.net) to predict the degree to which ARGs were shared between water and sediment samples and then determined the source of the ARGs. We used the water and sediment samples as the ARG source samples to train the model, respectively, and the remainder as the sink samples to test the model.

3. Results and discussion

3.1. Bacterial community structure

Based on the 16S rRNA gene analyses, the bacterial community compositions at the phylum level are shown in Fig. 1a. Overall, the *Actinobacteria* (28.5%), *Proteobacteria* (25.1%), *Bacteroidetes* (11.9%), *Chloroflexi* (8.8%), *Cyanobacteria* (8.6%) and *Acidobacteria* (5.7%) were the predominant phyla in the Danjiangkou Reservoir area. However, the predominant bacterial phyla in the water samples and sediment samples were quite different. *Actinobacteria* (40.2%), *Proteobacteria* (21.1%), *Bacteroidetes* (17.7%) and *Cyanobacteria* (13.7%) were the predominant phyla in water, while *Proteobacteria* (29.4%), *Chloroflexi* (16.9%), *Actinobacteria* (15.8%), *Acidobacteria* (11.3%) and *Bacteroidetes* (5.6%) were the predominant phyla in sediment. Some previous studies also observed differences in bacterial communities between water and sediment, and the main reasons for this are the different environmental factors between water and sediment, such as temperature, oxygen, nutrient, and light, which selected for different bacterial communities (Liu et al., 2018a; Walsh et al., 2016; Zinger et al., 2011).

The changes in the bacterial community at the phylum level in different seasons are shown in Fig. 1a. The relative abundances of the predominant phyla, such as *Actinobacteria* (37.4–43.1%), *Proteobacteria* (22.6–19.6%), *Bacteroidetes* (24.3–11.0%) and *Cyanobacteria* (12.0–15.4%), significantly changed in the water from the dry to the wet season. However, the relative abundances of predominant phyla, including *Proteobacteria* (29.0–29.8%), *Chloroflexi* (17.1–16.7%), *Actinobacteria* (15.7–15.9%), *Acidobacteria* (11.9–10.8%) and *Bacteroidetes* (5.7–5.5%), were almost constant in the sediment from the dry to the wet season (Fig. S2). Only some low-abundant phyla had slight changes between the two seasons, such as *Nitrospirae* (5.8–4.0%), *Firmicutes* (1.8–6.2%) and *Cyanobacteria* (4.7–1.5%), suggesting the bacterial community structures at the phylum level in the sediment were stable

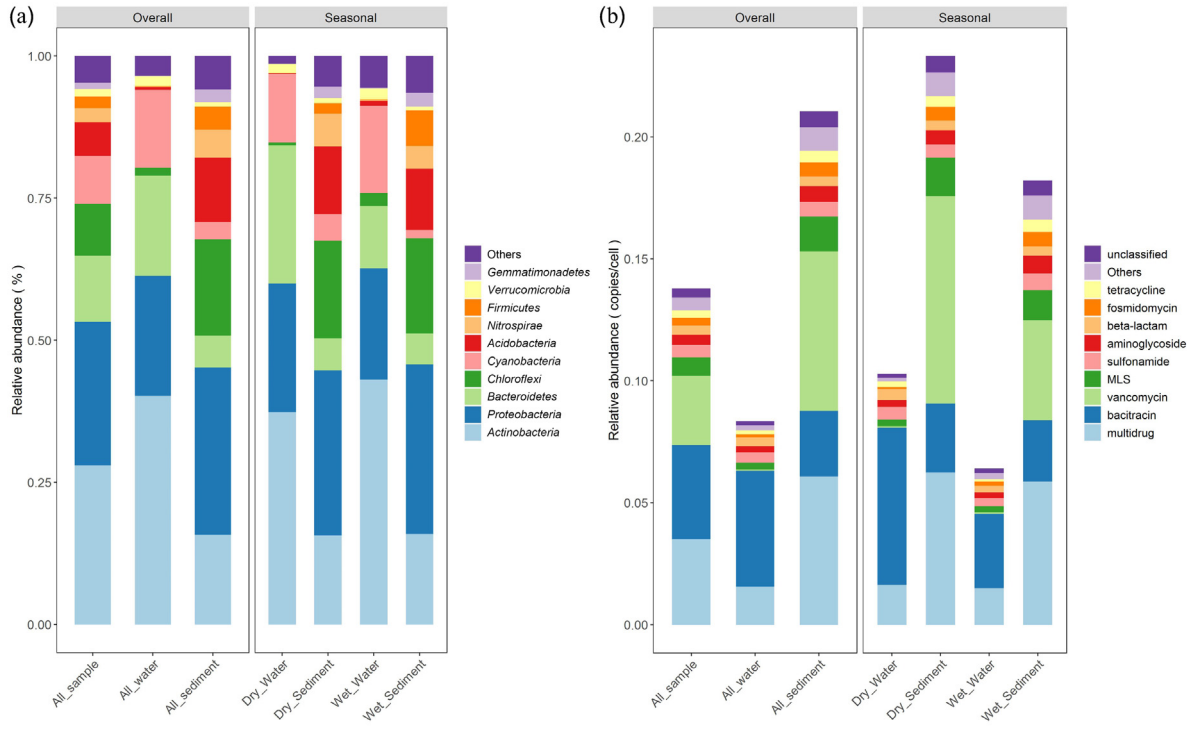

Fig. 1. The overall and seasonal profile of the bacterial community and ARGs in the water and sediment. (a) The relative abundance of sOTUs at the phylum level. (b) The relative abundance of ARG types, and the ARG numbers have been normalized with cell numbers. MLS: Macrolide-lincosamide-streptogramin.

across different seasons.

These results can be further supported by the PCoA plot based on the Bray-Curtis distances at the sOTUs level (Fig. 2a), and the bacterial communities from 4 groups (dry season water, dry season sediment, wet season water and wet season sediment) could be clustered into three parts. The bacterial communities in the water samples from the two seasons were clearly separated into two parts, but the communities in the sediment samples from the two seasons were clustered into one part. Based on the ANOSIM test, the communities in the water were significantly different between the dry and wet season (R = 0.9221, P = 0.001), whereas there were no significant dissimilarity for the sediment between the two seasons (R = 0.0113, P = 0.344) (Fig. S3). These results indicated that the bacterial communities in water were significantly affected by seasonal changes, but the communities in sediment were almost constant and hardly affected by seasonal changes. This result is consistent with previous studies that found that the seasonal fluctuation of bacterial communities in water is higher than that in sediment (Liu et al., 2018a). One possible explanation for this is that the characteristics of water (such as temperature, dissolved oxygen and flow discharge) significantly vary in response to seasonal changes, which ultimately results in differences in the bacterial community between seasons. As we expected, the key factors that shaped bacterial communities had strong seasonal fluctuations in the Danjiangkou Reservoir, and the average temperature (15.6–24.0 °C), dissolved oxygen (9.89–7.32 mg/L) and water level (150.5–153.9 m) had significant changes from the dry to the wet season (Table S1). Additionally, the

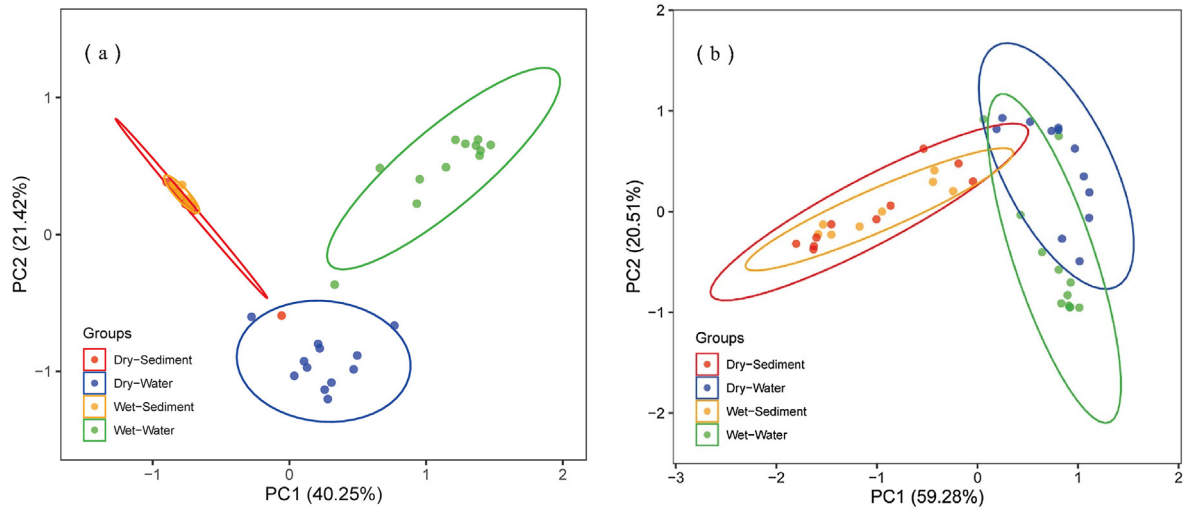

Fig. 2. Principal coordinate analysis (PCoA) plots show the composition differences (Bray-Curtis distances) of (a) sOTUs and (b) ARGs subtypes between water and sediment in different seasons. These groups are indicated by colored circles showing the 95% confidence intervals. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

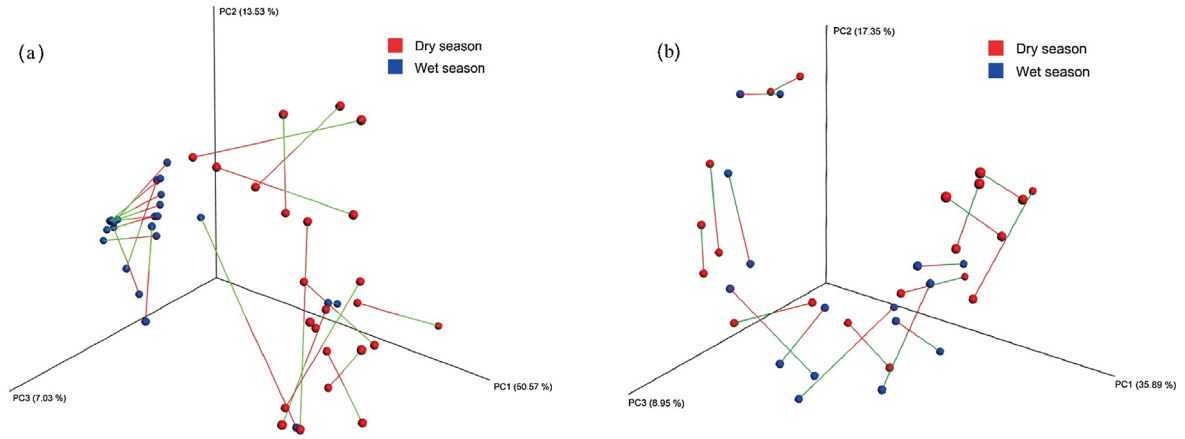

Fig. 3. The Procrustes analysis depicts the significant correlations between the bacterial community composition and ARG subtypes in (a) water ($M^2 = 0.518$, $P < 0.01$) and (b) sediment ($M^2 = 0.373$, $P < 0.01$).

sediment reached an equilibrium state through long-term processes of sediment erosion and deposition (Gibbons et al., 2014; Liu et al., 2018a), which led to a relative stable bacterial community.

3.2. The ARG abundance

In total, 436 ARG subtypes belonging to 20 ARG types were detected from 42 samples (Tables S3 and S4), with total abundances ranging from 0.032 to 0.409 copies/cell. The ARG type abundances (normalized by cell number) are shown in Fig. 1b. Overall, the average abundance of ARG types was 0.138 copies/cell. The bacitracin (0.039 copies/cell), multidrug (0.035 copies/cell) and vancomycin (0.028 copies/cell) resistance genes were the predominant ARG types. In water, the ARG type abundance was 0.083 copies/cell, and bacitracin (0.047 copies/cell) and multidrug (0.016 copies/cell) resistance genes were the predominant ARG types. In sediment, the ARG type abundance was 0.211 copies/cell, which was approximately 2.5 times that in water, and the vancomycin (0.066 copies/cell), multidrug (0.061 copies/cell), bacitracin (0.027 copies/cell) and macrolide-lincosamide-streptogramin (0.014 copies/cell) resistance genes were the predominant ARG types (Fig. 1b). Compared to other environments, the ARG abundance in the Danjiangkou Reservoir was comparable to that in some natural environments but lower than that in wastewater treatment plants or livestock farms (Table S5).

Fig. 1b shows the changes in ARG type abundances in the different seasons. In water, the abundances of ARG types in the dry season (0.103 copies/cell) were higher than those in the wet season (0.064 copies/cell). As the dominant ARG type, the bacitracin resistance gene decreased from 0.064 to 0.030 copies/cell, and another predominant ARG type, multidrug resistance gene, decreased from 0.016 to 0.015 copies/cell. In sediment, the abundances of ARG types in the dry season (0.233 copies/cell) were also higher than those in the wet season (0.182 copies/cell). Except for the vancomycin resistance gene, which was significantly reduced (0.085 to 0.041 copies/cell) from the dry to the wet season, the rest of the predominant ARG types, such as multidrug (0.062 to 0.059 copies/cell), bacitracin (0.028 to 0.025 copies/cell) and macrolide-lincosamide-streptogramin (0.016 to 0.012 copies/cell) resistance genes, had slight decreases. The higher ARG abundance in the dry season may be mainly related to higher antibiotic concentrations, as our previous study revealed that the total concentrations of antibiotics in the dry season were several times higher than those in the wet season (Li et al., 2019), which can exert a higher selective pressure on bacteria to enrich for ARB and ARGs. In addition, the longer water retention time in the dry season will exacerbate the enrichment of ARB and ARGs (Czekalski et al., 2015).

Moreover, PCoA based on the Bray-Curtis distances at the level of ARG subtypes was used to further reveal the compositional differences in ARGs in the two seasons (Fig. 2b). The 4 groups (dry season water, dry season sediment, wet season water and wet season sediment) could also be separated into three clusters. The ARG subtypes in water samples from the two seasons were separated into two clusters, whereas the ARG subtypes in sediment samples from the two seasons mixed together and became one cluster. The ANOSIM analysis also showed the ARG subtypes in water were significantly different between the two seasons ($R = 0.4552$, $P = 0.001$), and there were no significant differences in the composition of ARG subtypes in the sediment of the two seasons ($R = -0.0483$, $P = 0.679$) (Fig. S3). This pattern suggested that seasonal changes could significantly alter the ARGs compositions in water but had little impact on that in sediment.

Based on the above results, both the bacterial communities and ARG profiles in water were significantly influenced by seasonal changes, but those in sediment were almost constant and hardly affected by seasonal changes. However, the community compositional dissimilarities ($R = 0.9221$, $P = 0.001$) in water between the two seasons were greater than that of the ARGs ($R = 0.4552$, $P = 0.001$), which indicated that shifts in community were more sensitive than ARG profiles to seasonal changes. On the one hand, the variation in communities would cause the changes in ARGs (Forsberg et al., 2014, 2012), because the ARG-carrying bacterial communities were affected by seasonal changes. On the other hand, the occurrence of HGT between bacteria could disconnect the genes from bacteria (Ma et al., 2019), thus resulting in smaller dissimilarities in ARG profiles between the dry and wet seasons.

3.3. The horizontal gene transfer potential

Previous studies showed that the bacterial community structure determined the distribution of the resistome when the HGT frequency was not sufficient to obscure the association of ARGs with bacterial genomes, and a significant correlation between bacterial composition and ARG profiles could be observed (Forsberg et al., 2014; Ma et al., 2017). To estimate the HGT frequency in water and sediment, the Procrustes analysis based on the Bray-Curtis distances was performed to test the correlation between the microbial community at the sOTU level and the ARGs at the subtype level (Fig. 3). Visualized by the Procrustes analyses, both the ARG content and bacterial composition of the water clustered by season, consistently displaying highly significant goodness-of-fit measures by ANOSIM analysis. In contrast, the phylogenetic community composition of the sediment samples showed no variability between seasons. A significant correlation between the ARG profiles and bacterial composition were observed in both water ($P < 0.01$) and sediment ($P < 0.01$) samples, indicating the resistome in water and

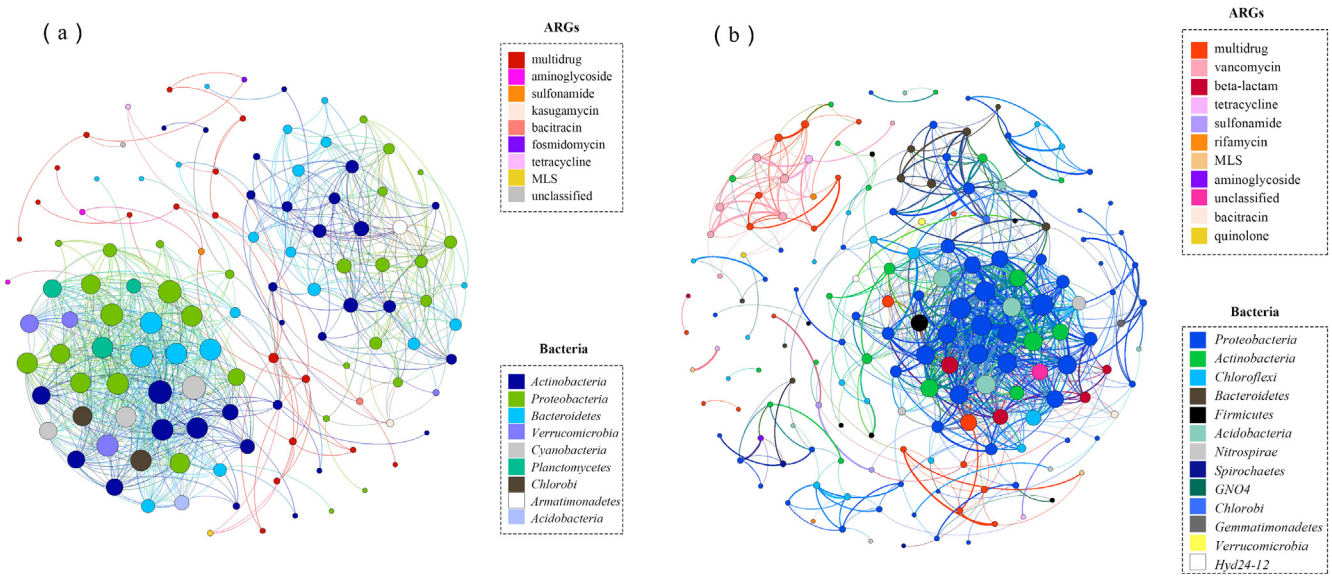

Fig. 4. The co-occurrence patterns between bacterial sOTUs and ARG subtypes in (a) water and (b) sediment. The nodes are colored according to the different ARG types or bacteria phyla. The size of each node is proportional to its number of connections, and a lager node indicates a stronger correlation with other points. A connection represents a strong (Spearman's correlation coefficients $\rho \geq 0.8$, P value ≤ 0.01) correlation. Panel (a) has 120 nodes and 837 edges, and 10 edges (1.2%) connect the ARG subtypes and sOTUs. Panel (b) has 176 nodes and 900 edges, and 168 edges (18.7%) connect the ARG subtypes and sOTUs.

sediment are both community-dependent. Although the ARG-HGT was not sufficiently frequent to obscure the association between ARGs and bacterial genomes in both water and sediment, it is interesting to note that the measure of fit (M^2 values) in water samples ($M^2 = 0.518$) was evidently higher than that in sediment samples ($M^2 = 0.373$), suggesting a higher tendency of ARG-HGT in the water microbiome (Ma et al., 2017). In addition, such a higher tendency of ARG-HGT in the water microbiome was also demonstrated by network analysis, where a weak correlation could be observed between ARG subtypes and bacterial sOTUs in water (Fig. 4a), while a strong positive correlation between resistance genes of multidrug, beta-lactam and vancomycin and sOTUs of *Proteobacteria, Actinobacteria* and *Acidobacteria* was found in a tightly clustered module of the sediment network (Fig. 4b).

Additionally, the total coverage of MGEs in the water was relatively higher in both the dry and wet season. For each sampling site, except for the DKKX and LSH sites, the coverage of MGEs in water was significantly higher than that in sediment in each season (Table S6), suggesting that the genes in water had greater HGT potential (Marti et al., 2014). We further screened the ARGs that co-occurred with MGEs (ARGs flanked with at least one MGE on the contig) to identify the mobilized ARGs (Ju et al., 2019), and compared the coverage of the mobilized ARGs in water and sediment. The mobilized ARGs were frequently detected in water, especially in dry season water but were only detected in a few sediment samples. The limited mobility of the sediment resistome may explain the strong positive correlation between ARGs and sOTUs (Forsberg et al., 2014), which also indicated that the HGT potential in sediment was lower than in water (Table S6). In addition, the coverage of mobilized ARGs in dry season samples was higher than that in the wet season (Table S6). These results suggested that the ARGs in the dry season were more prone to horizontal transfer compared to the wet season, which may pose a greater public health risk in the dry season, especially in water.

3.4. The ARG source

To examine the extent to which ARGs are shared between water and sediment samples, we generated rank abundance histograms of the 50 most abundant ARG subtypes. Fig. S4a shows the top 50 ARG subtypes in the water and sediment samples from the dry season; the ARG subtypes shared between water and sediment are depicted in black and the unique ARG subtypes are in red. Among the 50 most abundant ARG subtypes, 32 of them were shared between water and sediment in the dry season, and most of the shared ARG subtypes in the sediment were more abundant than those in the water, expect for bacitracin_bacA. For the wet season, the pattern of shared ARG subtypes was similar to that in the dry season (Fig. S4b). Our above results exhibited a significant within-site correlation between the ARG content and community composition in both the water and sediment (Fig. 3), and thus the ARG profile reflected the ARG-carrying bacterial community to a certain degree. Previous studies revealed that the bacterial community in sediment is able to spread to the water column when the abundance of shared bacteria in the sediment is much higher than its abundance in water (Walsh et al., 2016). Accordingly, a higher abundance of shared ARGs in sediment may also lead to spreading into the water.

In addition, we used SourceTracker to estimate the proportion of ARGs in water coming from sediment. As shown in Fig. 5, except for the water sample from the dry season at site BDT, all of the rest of the water samples were the ARG "sinks", and most of the ARGs in water came from the sediment, with the average proportion of the sediment source being as high as 94.4% and 89.2%, respectively, for the dry and wet seasons (Table S7). The sediment of sites DSQ and HK was the main source of ARGs in most of the water samples (except for site DJKBX), which accounted for an average of 79.5% and 70.1% of the total source in the dry and wet seasons, respectively. It is interesting to note that sites DSQ and HK were located in the upper reaches of the Dan and Han rivers, which both serve as the incoming rivers of the Danjiangkou Reservoir. However, the proportion of ARGs sourcing from the DSQ and HK sediment was reduced approximately 50% in the water of site DJKBX compared to the sites upstream of the dam, and the water resistome was mainly sourced from its sediment, accounting for 57.8% and 49.0% of the total source proportions in the dry and wet seasons, respectively (Table S7). This reduction in the influence of upstream sediment on the water resistome may be due to the presence of the Danjiangkou dam, which might act as a barrier for the dissemination of species and genes exchanged between upstream and downstream (Chai et al., 2009; Ruiz-Gonzalez et al., 2013; Wang et al., 2017).

We also tested the influence of water on the sediment resistome; Table S8 shows that the major source of ARGs in sediment is unknown. These results indicated that the water was the "sink" of ARGs and the sediment was the "source" of ARGs in the Danjiangkou Reservoir. As

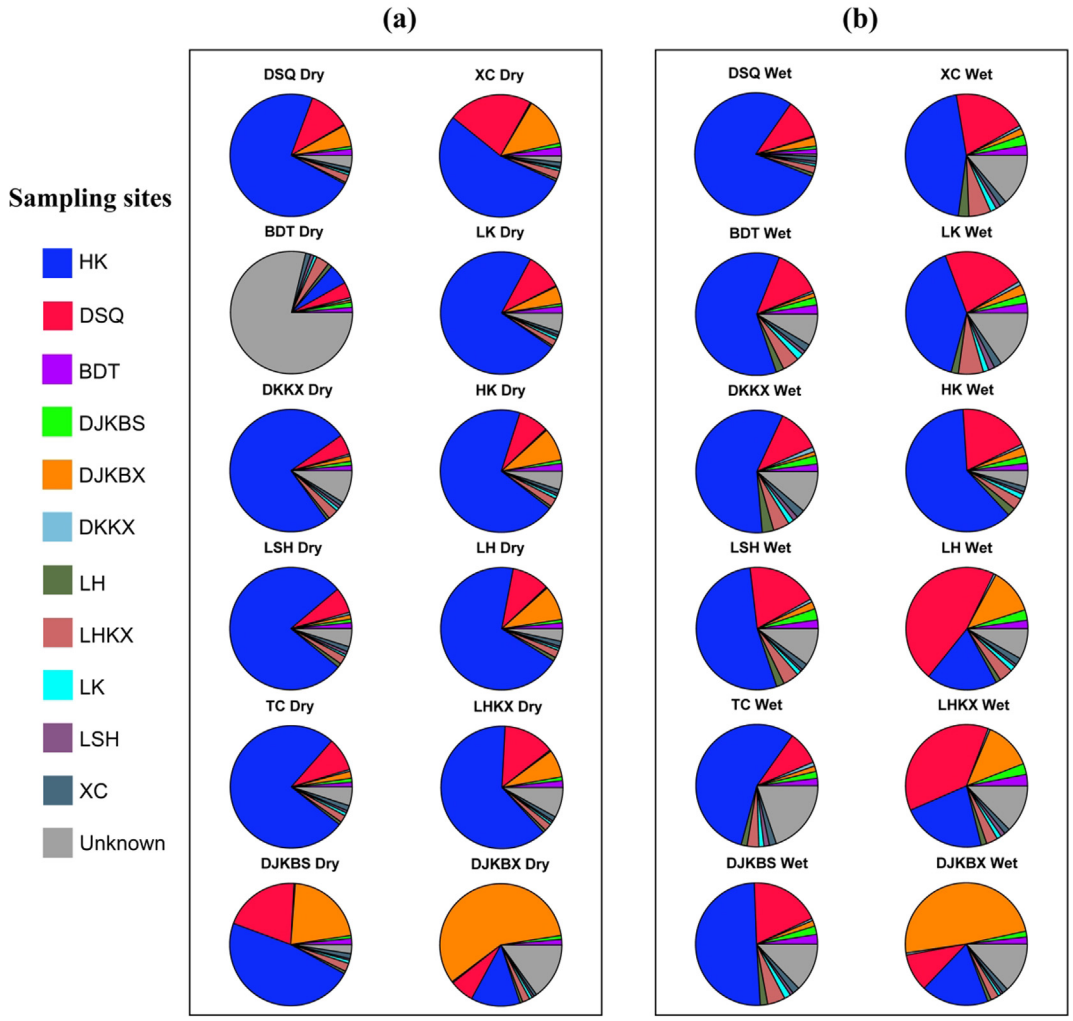

Fig. 5. The proportion of ARGs in water samples coming from sediment in the (a) dry season and (b) wet season. "Source tracker" shows the sediment was the estimated source of ARGs in the water. Each pie chart represents the composition of the sediment-derived ARGs in a water sample. The different colors represent the sediment at each sampling site, and "unknown" indicates the source cannot be determined. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

enforced by the protection act of the water source area in recent years, the government had shut down more than 800 factories upstream of the Danjiangkou Reservoir (The Five-Year Plan, 2017), which kept the external pollution input at a minimum rate such that the endogenous release from sediment became a major source of ARGs in Danjiangkou Reservoir.

3.5. The ARG hosts

We obtained a total of 12,567,853 contigs from 42 samples after filtering the contigs length < 500 bp, and 730 contigs were identified as ARG-carrying contigs through searching the ORF sequences against the SARG database (Table S9). Since the DJKBX site was not in the scope of the SNWDP water source area (the details are in Section 2.1 and Fig. S1), we ruled out this site to analyze the ARGs host and its potential risks. Finally, 509 ARG-carrying contigs were kept for ARG host identification and the subsequent analysis. We found that 419 of them (82.3%) were annotated at the phylum level, while only 207 of them (40.7%) were annotated at the genus level, which is the classification level comparable to previous work (Zeng et al., 2019). Overall, the coverage of ARG carriers in the dry season (24.67 copies/Gb) was higher than that in the wet season (7.43 copies/Gb) (Table S10), which is similar to the results of Section 3.2 based on the short reads.

Overall, *Proteobacteria* (44.5%) and *Actinobacteria* (32.8%) were the main ARGs carriers, whereas approximately 22.2% of ARG carriers were not annotated at the phylum level (Fig. 6). In water samples, *Burkholderiales* was the most frequently detected ARG carrier, accounting for 45.57% and 47.77%, respectively, of all ARG carriers in the dry and wet seasons, which mainly carried the resistance genes against bacitracin. Nevertheless, the most frequently detected ARG carrier in the sediment was *Actinomycetales*, mainly carrying the resistance genes against vancomycin, which accounted for 66.69% and 42.78%, respectively, in the dry and wet seasons (Fig. 6, Table S10). Similar to the results based on the short reads, bacitracin and vancomycin resistance genes were the predominant ARGs in water and sediment, respectively.

We identified 14 out of 509 ARG-carrying contigs as pathogens, including *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* (Table S11), and most of them (10/14 ARG-carrying pathogens) came from dry season samples. Moreover, 3 ARG-carrying *Escherichia coli* were also found to be carrying the virulence factor of polysialic acid capsule biosynthesis protein SiaD encoded by the siaD/synD gene. They were detected in the dry season: 2 from water samples and 1 from sediment samples. The polysialic acid capsule is the essential virulence factor that can cause lethal bacteremia and meningitis in humans invaded by certain strains of *Escherichia coli* and *Neisseria meningitidis*

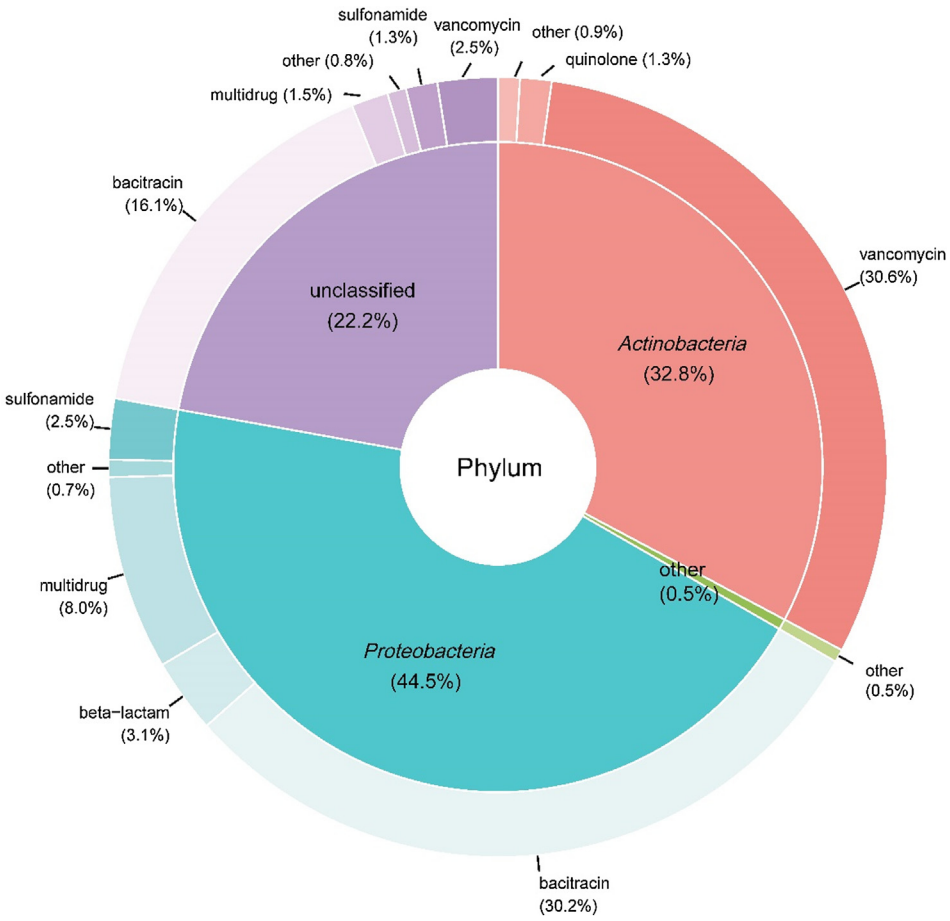

Fig. 6. The annotation information of the ARG-carrying contigs. The inner circle represents the annotation of the ARG host at the phylum level. The outer circle represents the composition of the ARG types.

(Ferrero and Aparicio, 2010; Steenbergen and Vimr, 2003). Previous studies revealed that *Escherichia coli* O157:H7 obtained the majority of its virulence factors from MGEs, and all *Escherichia coli* O157:H7 strains from clinical isolates possess a putative virulence plasmid named pO157 (Lim et al., 2010; Perna et al., 2001). In our study, we did not observe MGEs in close proximity to SiaD. One possible reason could be the mobility of virulence factors in these *Escherichia coli* strains was weak; or we might miss the MGE detections because the contigs were not long enough.

4. Conclusion

In this study, a total of 436 ARG subtypes belonging to 20 ARG types were detected from the 42 samples. The overall ARG abundance in the sediment was higher than that in the water, and bacitracin and vancomycin resistance genes were the predominant ARG types in water and sediment, respectively. The overall ARG abundance in the dry season was higher than that in the wet season, and a significant difference of ARG subtype compositions was observed in the water between the different seasons, but not in the sediment. The potential HGT frequency in the water was higher than that in the sediment, and the ARGs in the water mainly came from the sediment of sites DSQ and HK. *Burkholderiales* was the main ARG-carrier in the water samples, while *Actinomycetales* in the sediments composed the majority of the resistome. In addition, 14 ARG-carrying pathogens of *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* were detected, and 3 *Escherichia coli* carried virulence factors. Overall, the potential public health risks posed by resistomes in the water of the Danjiangkou Reservoir were higher in the dry season than in the wet season. Based on our results, the following management strategies are proposed to eliminate the potential threats to public health from ARGs in the Danjiangkou Reservoir: i. focus on controlling endogenous pollution from reservoir sediments; and ii. ARG-related factors should be considered for inclusion in assessments of water quality.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2019.105449.

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