Health risk ranking of antibiotic resistance genes in the Yangtze River

Jiang, Chunxia; Zhao, Zelong; Grossart, Hans Peter; Ju, Feng; Zhao, Yi; Gadd, Geoffrey Michael

DOI:
10.1016/j.ese.2024.100388

Publication date:
2024

Environmental Science and Ecotechnology 21 (2024) 100388

Contents lists available at ScienceDirect

Environmental Science and Ecotechnology

journal homepage: www.journals.elsevier.com/environmental-science-and-ecotechnology/

Original Research

Health risk ranking of antibiotic resistance genes in the Yangtze River

Chunxia Jiang [a, b, c], Zelong Zhao [d], Hans-Peter Grossart [e, f], Feng Ju [g], Yi Zhao [h], Geoffrey Michael Gadd [i, j], Ewa Korzeniewska [k], Yuyi Yang [a, b, c, *]

[a] Key Laboratory of Aquatic Botany and Watershed Ecology, Wuhan Botanical Garden, Chinese Academy of Sciences, Wuhan, 430074, China
[b] University of Chinese Academy of Sciences, Beijing, 100049, China
[c] Danjiangkou Wetland Ecosystem Field Scientific Observation and Research Station, Chinese Academy of Sciences & Hubei Province, Wuhan, 430074, China
[d] Liaoning Key Laboratory of Marine Fishery Molecular Biology, Liaoning Key Lab of Germplasm Improvement and Fine Seed Breeding of Marine Aquatic Animals, Liaoning Ocean and Fisheries Science Research Institute, Dalian, 116023, China
[e] Leibniz-Institute for Freshwater Ecology and Inland Fisheries (IGB), Neuglobsow, 16775, Germany
[f] Institute for Biochemistry and Biology, Potsdam University, Potsdam, 14469, Germany
[g] Key Laboratory of Coastal Environment and Resources of Zhejiang Province, School of Engineering, Westlake University, Hangzhou, 310030, China
[h] School of Water Resources and Environment, China University of Geosciences (Beijing), Beijing, 100083, China
[i] Geomicrobiology Group, School of Life Sciences, University of Dundee, Dundee, DD1 5EH, Scotland, UK
[j] State Key Laboratory of Heavy Oil Processing, State Key Laboratory of Petroleum Pollution Control, China University of Petroleum, Beijing, 102249, China
[k] Department of Water Protection Engineering and Environmental Microbiology, Faculty of Geoengineering, University of Warmia and Mazury in Olsztyn, Olsztyn, 10-720, Poland

ARTICLE INFO

Article history:
Received 1 July 2023
Received in revised form
2 January 2024
Accepted 2 January 2024

Keywords:
Antibiotic resistance genes
Yangtze River
Habitat
Coalescence
Health risk

ABSTRACT

Antibiotic resistance is an escalating global health concern, exacerbated by the pervasive presence of antibiotic resistance genes (ARGs) in natural environments. The Yangtze River, the world's third-longest river, traversing areas with intense human activities, presents a unique ecosystem for studying the impact of these genes on human health. Here, we explored ARGs in the Yangtze River, examining 204 samples from six distinct habitats of approximately 6000 km of the river, including free-living and particle-associated settings, surface and bottom sediments, and surface and bottom bank soils. Employing shotgun sequencing, we generated an average of 13.69 Gb reads per sample. Our findings revealed a significantly higher abundance and diversity of ARGs in water-borne bacteria compared to other habitats. A notable pattern of resistome coalescence was observed within similar habitat types. In addition, we developed a framework for ranking the risk of ARG and a corresponding method for calculating the risk index. Applying them, we identified water-borne bacteria as the highest contributors to health risks, and noted an increase in ARG risks in particle-associated bacteria correlating with heightened anthropogenic activities. Further analysis using a weighted ARG risk index pinpointed the Chengdu–Chongqing and Yangtze River Delta urban agglomerations as regions of elevated health risk. These insights provide a critical new perspective on ARG health risk assessment, highlighting the urgent need for strategies to mitigate the impact of ARGs on human health and to preserve the ecological and economic sustainability of the Yangtze River for future human use.

1. Introduction

Antimicrobial resistance (AMR) was declared a global public health threat by the World Health Organization (WHO) [1]. It is predicted to cause up to ten million deaths and $6.1 trillion in losses globally each year by 2050 if no action is taken [2,3]. Evidence shows that some clinical antibiotic resistance genes (ARGs) originated in environmental organisms [4,5]. As one of the largest biodiversity reservoirs, rivers are home to various ecological processes, yet rivers are highly vulnerable to anthropogenic pollution from many different sources [6–9]. Environmental bacteria can acquire antibiotic resistance through horizontal gene transfer (HGT) and mutation [10]. Meanwhile, the resistance acquisition can

* Corresponding author. Key Laboratory of Aquatic Botany and Watershed Ecology, Wuhan Botanical Garden, Chinese Academy of Sciences, Wuhan, 430074, China.
E-mail address: yangyy@wbgcas.cn (Y. Yang).

https://doi.org/10.1016/j.ese.2024.100388

be accelerated due to anthropogenic stressors [11,12].

The Yangtze River is one of the most important freshwater sources in China; consequently, its antibiotic-resistance pollution is of great public health concern. Studies have shown that ARG occurrence varies greatly between riverine ecosystems, whereby anthropogenic activities are important drivers of antibiotic resistance pollution [13–15]. The Yangtze River is the third longest river in the world (6300 km) and has different land use types along its banks. Therefore, different sections of the river are subjected to different anthropogenic disturbances. In addition, river ecosystems represent a continuum of interconnected habitats that extend longitudinally, laterally, and vertically, with significant changes in biological characteristics [16]. This may affect the distribution and coalescence of antibiotic resistance in different riverine habitats. Wang et al. studied the ARG hosts throughout the Yangtze River basin using a metagenomic assembly approach [17]. However, the coalescence and risk quantification of ARGs in different habitats of the Yangtze River are still not studied.

In previous river studies, the potential mobility, host pathogenicity, and co-occurrence with virulence factors of ARGs were obtained by metagenomic assembly approach to assess the risk of ARGs [18,19]. However, these methods are unable to accomplish the risk quantification. In addition, studies have shown that not all ARGs exhibit health risks to humans, and ARG abundance is not sufficient to represent ARG health risk [20,21]. In recent years, researchers have provided insights into ARG risk assessment from different perspectives [21–24]. However, these methods have disadvantages, such as the limitations of the samples used to develop the risk assessment methods and the inability of current metagenomic assembly technology to cover all the sequences. To overcome these limitations, we constructed a new risk ranking ARG framework and proposed a practical method for risk quantification.

In our study, (i) we provide the first comprehensive evaluation of ARGs in six habitats (free-living setting, particle-associated setting, surface sediment, bottom sediment, surface bank soil, and bottom bank soil) along the Yangtze River; (ii) the resistome coalescence and hosts of the Yangtze River; (iii) a new ARG risk ranking framework to assess the ARG risks along the Yangtze River. This study provided the first resistome evaluation along the Yangtze River watershed and expanded current ARG risk analysis.

2. Materials and methods

2.1. Sampling sites and sample collection

To assess the pollution of ARGs in the Yangtze River ecosystem, we conducted a sampling campaign in October and November 2019. A total of 204 samples were collected from 37 sampling sites (Fig. S1) across approximately 6000 km of the Yangtze River, with each site including six different habitats: free-living setting (WF), particle-associated setting (WP), surface sediment (SES), bottom sediment (SEB), surface bank soil (BSS), and bottom bank soil (BSB).

In brief, composite water samples (6 L) were collected and filtered sequentially through 2.0 μm and 0.22 μm Durapore membrane filters (Merck Millipore, Watford, UK) to obtain particle-associated and free-living bacterial communities, respectively [25]. Surface sediment and bank soil samples were collected from 0 to 5 cm depth; bottom sediment and bank soil samples were collected from 10 to 15 cm depth. Each composite sample consisted of three subsamples; the composite sample was subsequently divided into two parts, one for genomic analysis and one for environmental variable detection. The samples for environmental variable detection were stored at 4 °C, and the samples for genomic analysis were stored at −20 °C. The basic information for all samples is given in Table S1.

2.2. Environmental variables and anthropogenic activities

Environmental variables of the soil or sediment, including total carbon (TC), organic carbon (TOC), ammonium (NH_4^+-N), nitrate (NO_3^--N), total nitrogen (TN), total phosphorus (TP), available phosphorus (AP), available iron (AFe), available copper (ACu), available zinc (AZn), and available manganese (AMn) were determined using standard analytical methods [26–28]. Environmental variables of water, including NH_4^+-N, nitrite (NO_2^--N), NO_3^--N, TN, TP, TC, TOC, pH, electrical conductivity (EC), salinity, oxidation-reduction potential (ORP), dissolved oxygen (DO), and temperature were also measured [29,30].

Anthropogenic factors (i.e., socio-economic factors: population density, gross domestic product (GDP), urbanization rate, gross output value of fishery, residential and industrial area, artificial aquaculture production, number of patients diagnosed and treated, and number of hospitalized patients) of the administrative regions in which the sample sites are located were obtained from the Provincial Bureau of Statistics and the Chinese National Bureau of Statistics (http://www.stats.gov.cn/). Then, all anthropogenic factors were normalized by the area of administrative regions (km^2) in which the sampling sites are located.

2.3. Total genomic DNA extraction and sequencing

After extraction using HiPure Soil DNA Kits (Magen, Guangzhou, China), genomic DNA was estimated using a Nanodrop 2000 spectrophotometer (Thermo Fisher Scientific Inc., Waltham, US). Paired-end (2 × 150 bp) sequencing reads were generated on the Illumina Novaseq 6000 platform (Novogene, Beijing, China), yielding approximately 2792.6 Gb reads, with an average of 13.69 Gb for each sample (Table S2). To ensure the quality of downstream analysis, metagenomic datasets (raw data) were quality controlled using Trimmomatic 0.40 to remove splice contaminants and low-quality sequences with the specific parameters (ILLUMINACLIP:merge.fa:2:30:10 SLIDINGWINDOW:4:15 MINLEN:75) [31]. Reads that passed quality control were then mapped to the human genome (version: hg19) using the BWA mem algorithm (parameters: M -k 32 -t 16, http://bio-bwa.sourceforge.net/bwa.shtml) to remove possible human genome contamination. As a result, a total of 2684.21 Gb and an average of 13.16 Gb sequence data (clean reads) were used for subsequent analysis (Table S2).

2.4. Identification of ARGs and taxonomic annotation

The retrieved clean reads were used to search for ARGs and MGEs using the online analysis pipeline ARGs-OAP v2.0 [32] with the SARG and a custom MGE (https://github.com/KatariinaParnanen/MobileGeneticElementDatabase) [33] database, respectively.

In brief, potential ARG, MGE, and bacterial 16S rRNA sequences from the metagenomic datasets were pre-screened potential ARGs sequences from short reads in metagenomic datasets using UBLAST on a local computer, followed by a second step in which ARGs were annotated and classified online using BLASTX with the e-value at 1×10^{-5} after uploading identified potential ARGs sequences [34]. The sequence was considered to be an ARGs-like sequence when its best hit had a similarity of no less than 80% to the reference sequences and had a query coverage of no less than 25 amino acids [35]. To avoid potential biases during cross-sample comparisons, ARG abundance was normalized by the length of the reference sequences. ARG and MGE abundance units were converted to copy number per 16S rRNA gene copy number (copies per 16S rRNA gene) [36]. The taxonomy of clean reads was determined by Kraken2 [37] using the customized Kraken database, including

bacteria, archaea, viruses, and eukaryotes. Only reads classified as bacteria were retained, and then the abundance of each taxonomy was corrected by Bracken [38].

2.5. Metagenome-assembled genomes and annotation

Contigs were generated using MEGAHIT with "—min-contig-len 500" parameters [39]. Contigs were then binned and clustered using metaBAT2 [40] to obtain metagenome-assembled genomes (MAGs). The completeness and contamination of all MAGs were obtained using CheckM v.1.0.3 [41]. MAGs with >50% completeness and <10% contamination were retained. To improve the assembly quality of MAGs, we used the bwa mem function of metaSPAdes [42] for MAGs reassembly after extracting data from clean reads. The abundance of each MAG was then calculated using the metawrap quant_bins module [43].

The open reading frames (ORFs) in each MAG were predicted using Prodigal (v2.6.3) [44]. We used the BLASTX matching function in ARGs-OAP v2.0 for ARG annotation, ORFs with >50% similarity and >150 bp alignment length to the reference sequences in SARG were defined as antibiotic-resistant ORFs (i.e., ARGs) [32,45]. The MAGs carrying ARGs were picked out, and included ORFs were also matched against the customized MGE database [33] by using the BLASTX in ARG-OAP v2.0 to identify the co-occurrence of ARGs and MGEs. Taxonomic annotation of MAGs carrying ARGs was performed using GTDB-Tk (v0.2.2) [46]. To facilitate comparisons between samples, MAG abundance was normalized using Kallisto (v0.46.2) [47].

2.6. ARG risk ranking

A new metagenomic-based approach for the health risk ranking of ARGs was developed in this study. We defined ARGs occurring in the genomes of bacteria associated with human diseases as pathogenic and used an ARG database with acquired ARGs to discriminate ARG mobility. To achieve these goals, two main databases were used in this study: the Pathosystems Resource Integration Center (PATRIC) (https://www.patricbrc.org) [48], and the ResFinder database (https://cge.food.dtu.dk/services/ResFinder/) [10]. The framework details are present in Supplementary material 1: Text S1 and Fig. S2a.

2.7. Statistical analysis of data

Analysis of variance (ANOVA) was performed using SPSS 17.0 to assess the difference between the means of various analysis results among different groups. The distance-decay similarity of ARG abundance across different habitats was assessed by linear regression between the Bray-Curtis distance of ARG composition and dendritic distance among two samples from each habitat. We calculated the pairwise distances between the ARGs (abundance and composition) and each environmental variable and performed Mantel tests using the ggcor package in R. Non-metric multidimensional scaling (NMDS) based on the Bray-Curtis distance was used to assess differences in the ARG composition across subgroups of the sample, significance in differences across subgroups was tested using analysis of similarity (ANOSIM). Venn analysis was performed to identify shared and unique ARGs of each of the six sampled habitats. To investigate the coalescence of ARGs across different habitats in the Yangtze River, we quantified the intensity of ARG interaction using overlapped ARG identification and SourceTracker (http://sourcetracker.sf.net) [49]. In the overlapped ARG identification, different habitats at each sampling site were considered sources and destinations for resistome coalescence. The SourceTracker analysis was performed with one given habitat as a sink and the other five habitats as sources, and then the source of the ARGs was determined.

Network analysis was used to investigate the co-occurrence patterns of ARGs in the different habitats. Spearman correlation was used to calculate all possible pairwise correlation coefficients (ρ) between ARG subtypes, and results with $\rho > 0.8$ and $P < 0.01$ were retained. To reduce the probability of false positives in the results, the Benjamini-Hochberg method [50] was used in this study to adjust the P values. Pairwise correlations between ARGs formed their co-occurrence networks, and Gephi software (v.0.9.2) was used to visualize co-occurrence networks [51]. The association of ARG-MGE or ARG-bacteria was assessed by Procrustes analysis (based on principal coordinates analysis (PCoA) with Bray-Curtis distance), with $P < 0.05$ indicating significant correlation and $P < 0.01$ indicating a highly significant correlation. PERMANOVA was used to assess the effect of anthropogenic activities on the abundance of risky ARGs. Linear correlation analysis was used to confirm the relationship between anthropogenic activities and the weighted index of ARG risk.

3. Results

3.1. Broad-spectrum resistome profiles in the Yangtze River

Twenty-two types of ARGs (655 ARG subtypes) constituted the whole resistome of the six sampled riverine habitats (free-living setting, particle-associated setting, surface sediment, bottom sediment, surface bank soil, and bottom bank soil) (Fig. 1a and b). ARGs in free-living (518 ARG subtypes, 0.26 copies per 16S rRNA gene) and particle-associated (554 ARG subtypes, 0.25 copies per 16S rRNA gene) settings were the most diverse and abundant (one-way ANOVA, $P < 0.05$) (Fig. 1a,c). We observed that ARG similarity decreased as the dendritic distance increased (Fig. S3). In addition, ARGs in different habitats were positively correlated with different sets of environmental factors; ARGs in free-living and particle-associated settings were related to most environmental factors (Fig. S4). Consequently, ARG occurrence can be influenced by habitat type, geographical separation, and environmental conditions.

Beta-lactam-ARGs showed the highest number of subtypes in each habitat (Fig. 1b), but at most sampling sites, their proportion was lower than multidrug-ARGs (Fig. S5a). This is possibly related to existing variable subtypes of beta-lactam-ARGs along the Yangtze River. As shown in Fig. 1d and Fig. S5b, the genes for bacitracin, macrolide-lincosamide-streptogramin (MLS), sulfonamide, vancomycin, and multidrug resistance formed the major part of the Yangtze River resistome. Yet, vancomycin-ARGs only occurred in low abundance in free-living (0.844—5.128 copies per 16S rRNA gene) and particle-associated (0.995—6.400 copies per 16S rRNA gene) settings. Chloramphenicol- and sulfonamide-ARGs in free-living and particle-associated settings were one order of magnitude higher in abundance than in the other studied habitats (Fig. 1d). In addition, our results suggest that different ARG types may exhibit different habitat affinities (Fig. S6).

The free-living and particle-associated settings had more unique ARGs and were distinctly separated from the other habitats (Fig. 1e—g). In addition, the sampled habitats revealed different core resistomes (occurring at all sampling sites in a specific habitat), and 22 core ARG subtypes were detected across all samples (Fig. S7). Multidrug-ARGs formed the main part of the core resistome in the study area. There were higher numbers and abundances but lower proportions of core resistomes in free-living and particle-associated settings than in all other habitats (Fig. S8). Other than the ARG habitat affinities, relatively frequent ARG exchange between sample sites of the two water-associated habitats

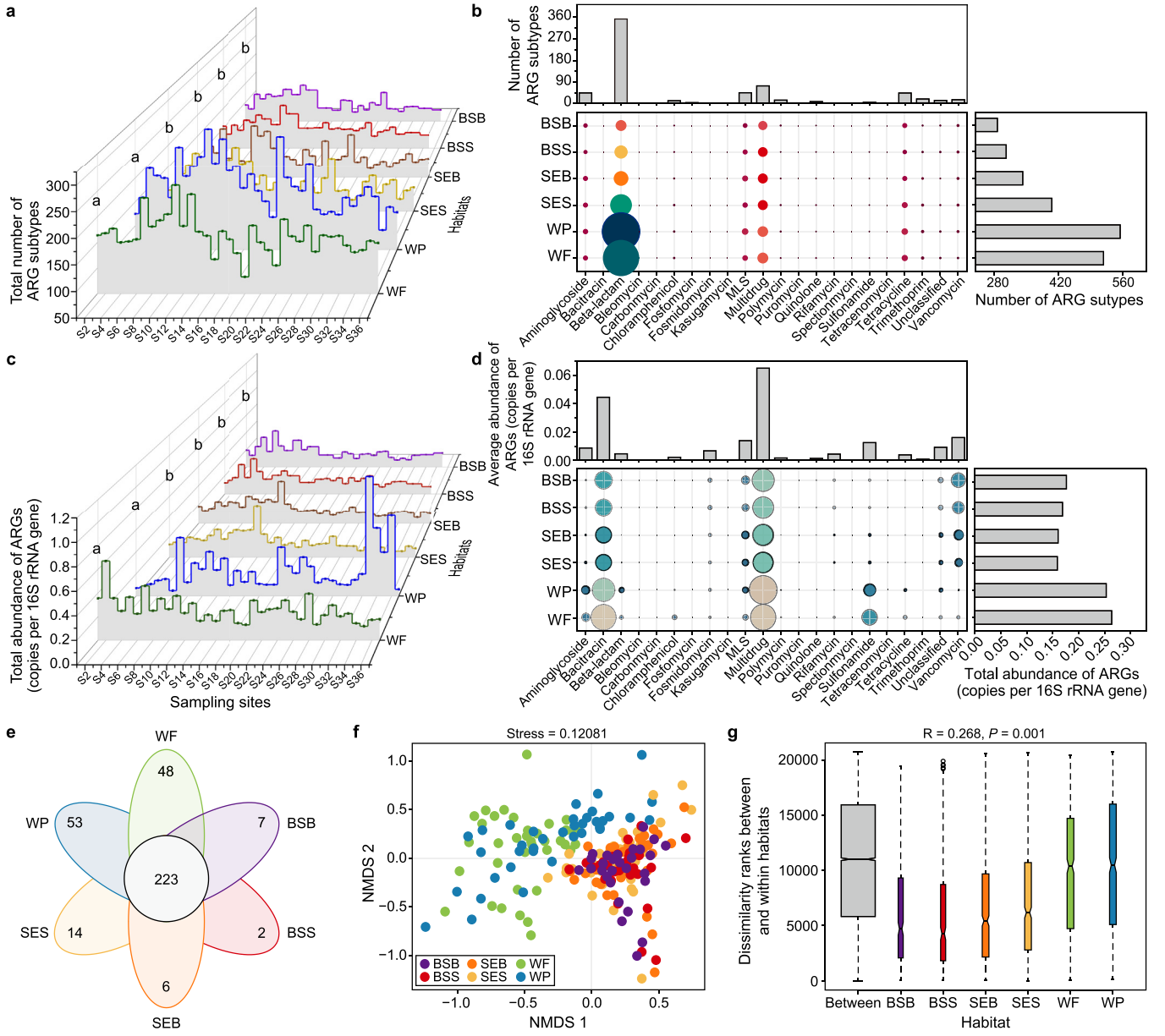

Fig. 1. Antibiotic resistance gene (ARG) distribution patterns in the Yangtze River. **a, c,** ARG number (**a**) and abundance (**c**) of different sampling sites. Different letters denote significant differences between habitats (shown in the legend) at the 0.05 probability level based on ANOVA. **b, d,** ARG number (**b**) and abundance (**d**) of different habitats. **e**, Shared and unique ARGs between the sampled habitats. **f**, Non-metric multidimensional scaling (NMDS) of ARGs between habitats. **g**, Dissimilarity ranks of ARGs between and within habitats. WF: free-living setting; WP: particle-associated setting; SES: surface sediment; SEB: bottom sediment; BSS: surface bank soil; BSB: bottom bank soil; MLS: macrolide-lincosamide-streptogramin.

may be another reason for this phenomenon. This conjecture was further confirmed by the more complex ARG-ARG clustering network of the water habitats (more edges and nodes in them than the others) (Fig. S9 and Table S3).

3.2. Resistome coalescence in the Yangtze River

As with many biological pollutants, the migration of ARGs also affects their occurrence and functional expression in the habitat. To investigate the coalescence of ARGs across different habitats in the Yangtze River, we used two methods, i.e., overlapped ARG identification and SourceTracker [52].

Different habitats at each sampling site were regarded as sources and destinations for resistome coalescence. To quantify ARG coalescence across habitats at each sampling site, we determined the overlapped ARGs of all sampling sites along the Yangtze River. Our results clearly show that the intensity of ARG coalescence fluctuated across the sampling sites (Fig. 2a). The variable proportion of overlapped ARGs in the study area may be associated with the variations in microbial communities [53], HGT [54], and pollutant sources [55] between the different sampling sites. We then calculated the overlapped ARGs between two individual habitats. The results revealed that proportions of overlapped ARGs between the same habitat type (i.e., WF-WP, SES-SEB, SES-BSS, SES-BSB, SEB-BSS, SEB-BSB, and BSS-BSB) were significantly higher than of overlapped ARGs between any other combination of paired

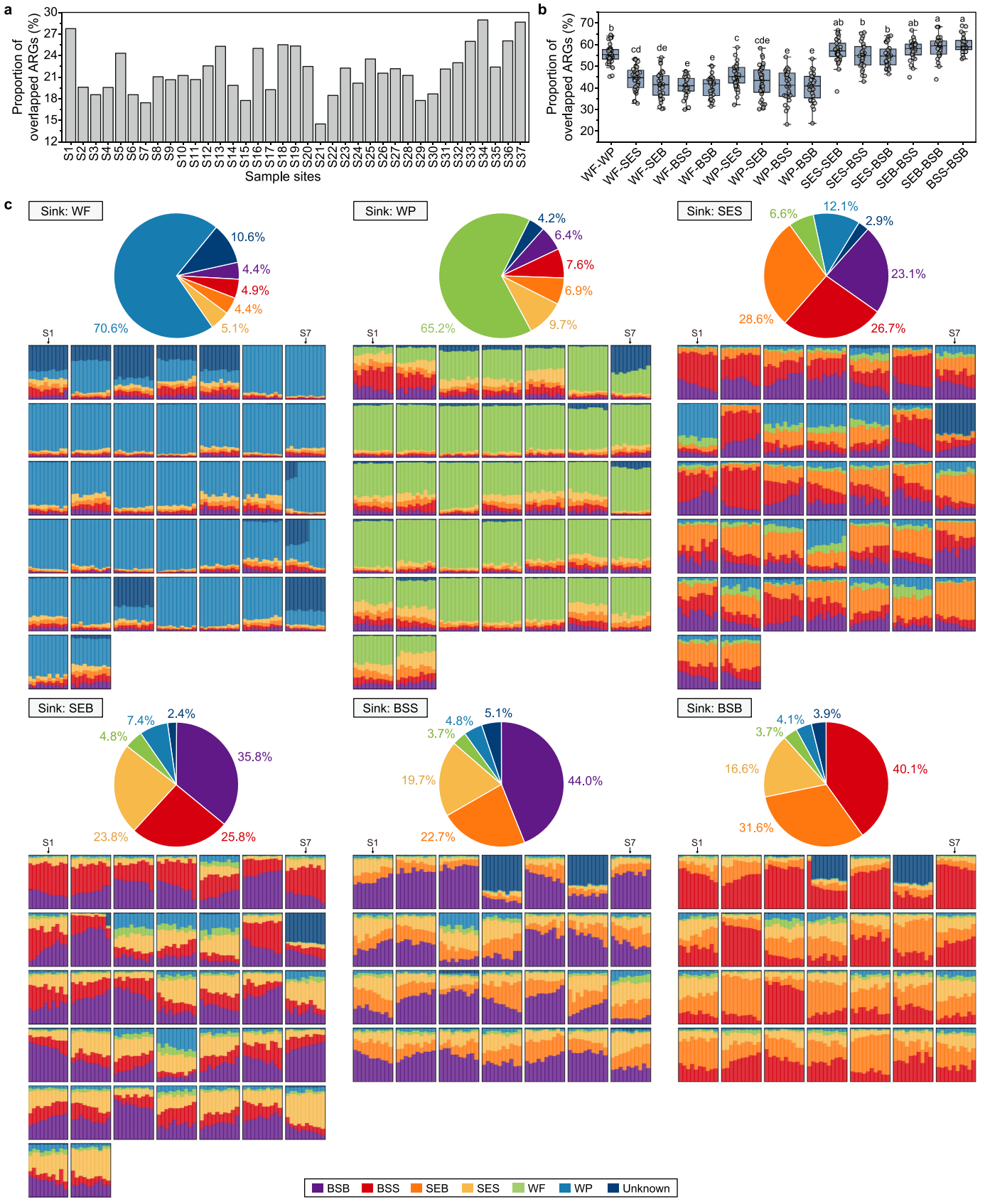

Fig. 2. Coalescence of the Yangtze River resistomes. **a**, Proportions of overlapped ARGs to overall ARGs in any paired habitats. **b**, Proportions of overlapped ARGs to overall ARGs in all habitats of each sampling site. Different letters denote significant differences between groups (shown in the legend) at the 0.05 probability level based on ANOVA. **c**, Proportion of ARGs in one given habitat comes from the other five habitats. The stacked plots in the graph represent the sources of ARGs at each of the 37 sampling sites labeled S1–S37. The first stacked plot on the top left corner of the graph (Row 1, Column 1) represents Sample S1, and the remaining stacked plots are arranged in a left-to-right, top-to-bottom order. The plots of S9, S13, S14, S16, S31, S33, S34, S36, and S37 in BSS and BSB are not shown. All abbreviations are the same as in Fig. 1.

habitats ($P < 0.05$) (Fig. 2b). In addition, the proportion of overlapped ARGs between water-associated habitats and bank soils was the lowest in this study, which may be due to the relatively restricted exchange between water and bank soil.

To further analyze and quantify ARG coalescence between different habitats, we mined our sequence data using SourceTracker, which is based on a Bayesian classifier (Fig. 2c). Results show that the highest proportion of the mutual ARG contribution occurred between free-living and particle-associated settings (WF to WP: 70.6%; WP to WF: 65.2%). Two sedimentary habitats showed a higher proportion of mutual contributions in the middle and lower reaches than in the upper reaches of the Yangtze River. Sediments of the middle and lower reaches of the Yangtze River also showed a high contribution to the bank soil resistome, which was higher than in the upper reaches. In this study, only a few sample sites showed a large proportion of unknown ARG sources, suggesting that there may be stationary and similar ARG sources along the entire Yangtze River. Meanwhile, the higher ARG abundance and diversity in free-living and particle-associated settings than in all other habitats imply that water-associated habitats may be in tighter contact with external ARG sources and/or fluctuating environmental conditions.

3.3. ARG mobility and hosts in the Yangtze River

Genes with high similarity to those of clinically relevant antibiotic-resistant pathogens have been identified in natural resistomes. Understanding the mobility and host relationships of environmental ARGs are key steps in dissecting resistomes.

Procrustes analysis revealed that ARGs were significantly correlated with MGEs in all habitats except bottom bank soil (Fig. S10). This tight correlation between ARGs and MGEs suggests that ARGs in the particle-associated setting and surface bank soil may be controlled by HGT. To precisely prove the co-occurrence patterns of ARGs and MGEs, we studied gene contexts based on MAGs in detail. We found a co-occurrence of ARGs and MGEs in 53 MAGs, of which 46 were from Proteobacteria (Table S4). ARGs and MGEs in these MAGs were mainly multidrug-ARGs and transposase genes. Hereafter, 13 MAGs were selected for gene context visualization in Fig. S11. *Mac*B and other MLS-ARGs mostly appeared in the same MAG together with integrase genes, and aminoglycoside-ARGs appeared in multiple co-occurrences with transposase genes. This indicates HGT may partially control the spread of ARG among bacteria in the Yangtze River.

As visualized by Procrustes analysis, both ARGs and bacterial communities of the six sampled habitats clustered by samples and consistently displayed highly significant goodness-of-fit measures (Fig. S12). In addition, we identified 127 MAGs as ARG hosts in all studied samples. These MAGs were from 23 bacterial phyla, mainly classified as Gammaproteobacteria (23.88%) and Alphaproteobacteria (17.16%) (Fig. S13), which often include antibiotic producers or bacteria with the capacity to transform or metabolize antibiotics [20]. The ten most prominent ARGs with more complex host relationships were *mac*B, *bac*A, *tet*P, *ksg*A, *ros*A, *ros*B, *mdt*B, *mdt*C, *mex*F, and multidrug ABC transporter (Fig. S14). Furthermore, we matched the identified ARG hosts with the pathogens prioritized by the Robert Koch Institute in Germany and the most relevant drug-resistant organisms according to the WHO list [56] and found no hits.

Notably, though MAG assembly can yield strain-level information, it is usually at the expense of fewer binned reads [57] potentially excluding ARGs of low abundance. Therefore, "reads"-based ARGs may provide a true picture of their risk to human health.

3.4. ARG risk assessment in the Yangtze River

The risk ranking results of ARGs in the SARG database (Supplementary material 1: Text S2, Figs. S2b and c, and Fig. S15; Supplementary material 2: Tables S5–S7) were used in the ARG health risk assessment of the samples from the Yangtze River (Supplementary material 2: Table S8). We found that the abundance of ARGs in different risk ranks varied between the sampling sites, but the risky ARGs (R1, R2, R3) showed a consistently higher abundance in most areas with pronounced anthropogenic activities (Fig. 3a). At sample sites S1–S4, R1 ARGs of free-living settings showed a higher abundance than at other sample sites, while the composition of R1 ARGs at these four sample sites was significantly different from the rest (i.e., chloramphenicol-ARGs were dominant) (Fig. S16). Compared to ARGs in the other two risky ARG ranks, the composition of R2 ARGs was relatively consistent among all habitats of the Yangtze River, with the sole difference that larger proportions of R2 sulfonamide-ARGs and aminoglycoside-ARGs were present in the free-living and particle-associated settings than in other habitats. In contrast, R3 ARGs showed different spatial distribution patterns and compositions in free-living and particle-associated settings (Fig. S16).

Difference analysis of specific risk rank ARGs revealed that R1, R2, and R3 ARGs were significantly more abundant in free-living and particle-associated settings than in any of the four other habitats (Fig. 3c). In addition, ARG risk ranking patterns in the sampled habitats showed an obvious dissimilarity, but risk-free ARGs were consistently dominant in all habitats (Fig. 3a and b).

Cluster analysis of sampling sites based on three of the presented risky ARG ranks revealed that different risky ARG ranks exhibited diverse patterns of geographical clustering (Fig. S17). Subsequently, to quantify the risk of ARGs for individual sampling sites along the Yangtze River, we calculated the weighted index of ARG risk for each sample (Fig. 4). It is obvious that ARGs showed relatively high health risks in the Chengdu-Chongqing and the Yangtze River Delta urban agglomeration. Sampling site S33 posed the highest ARG risk (0.355) of the entire study area. Meanwhile, our results revealed that the contribution of free-living and particle-associated bacteria (water-borne bacteria) to the overall ARG health risk was greater than that of bacteria of all other habitats.

We analyzed the relationship between risky ARG abundance and anthropogenic activities (Table S9) and found that anthropogenic activities constituted significant factors influencing risky ARGs in the sediments and bank soils, while they had little impact on risky ARGs in the waters. Additionally, we found that urbanization rate and gross output value of fishery were the dominant anthropogenic factors driving risky ARG abundance. Next, we dissected the relationship between the weighted index of ARG risks and anthropogenic activities (Fig. S18). We found that only ARG risk for particle-associated bacteria was positively associated with anthropogenic disturbances.

4. Discussion

4.1. Broad spectrum of resistome

With increased urbanization, industrialization, and heavy discharge of anthropogenic waste containing ARGs and antibiotic-resistant bacteria (ARB), rivers are becoming major reservoirs of antibiotic resistance [55]. Humans are at risk of being directly exposed to heavily contaminated river water. Thus, it is urgent to uncover the fate of ARGs in riverine ecosystems. In this study, a total of 655 subtypes of ARGs were identified, which was higher than that of the Yarlung Tsangpo River (89 subtypes) [58], the Ili River

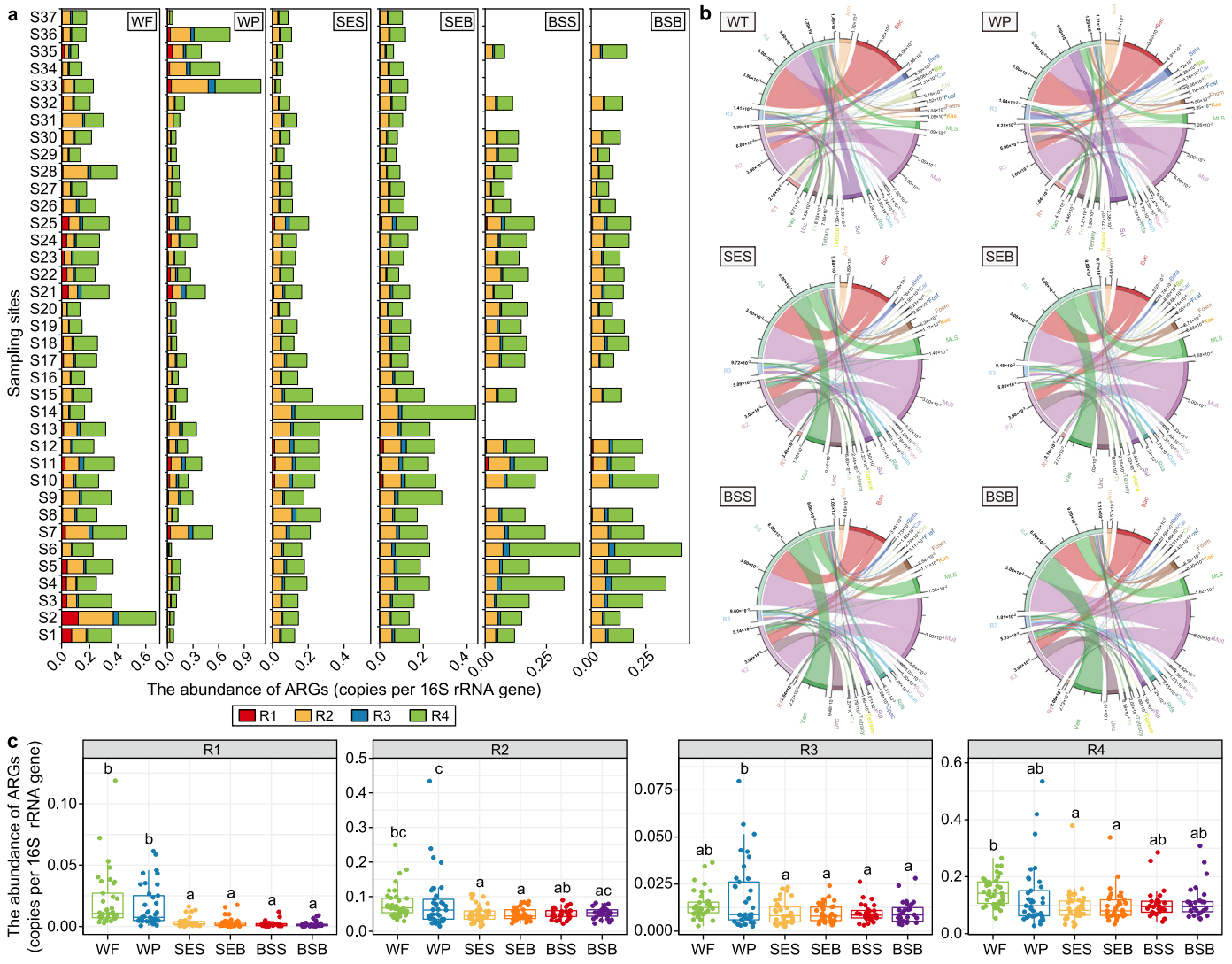

Fig. 3. Distribution patterns of different risk rank ARGs. **a**, Abundance of ARG risk rank groups in the sampled habitats. **b**, Resistome profiles of different risk rank groups. **c**, Difference analysis of specific ARG risk rank groups between the sampled habitats. Different letters denote significant differences between habitats (shown in the legend) at the 0.05 probability level based on ANOVA. Ami: Aminoglycoside; Bac: Bacitracin; Beta: Beta-lactam; Ble: Bleomycin; Car: Carbomycin; Chl: Chloramphenicol; Fosf: Fosfomycin; Fosm: Fosmidomycin; Kas: Kasugamycin; Mult: Multidrug; Poly: Polymyxin; Puro: Puromycin; Quin: Quinolone; Rifa: Rifamycin; Spec: Spectionmycin; Sul: Sulfonamide; Tetrace: Tetracenomycin; Tetracy: Tetracycline; Tri: Trimethoprim; Unc: Unclassified; Van: Vancomycin. Other abbreviations are the same as in Fig. 1.

(274 subtypes) [59], and the Beijiang River system in the Pearl River Basin (135 subtypes) [60]. These ARGs encompass almost all types of antibiotics. This suggests that the Yangtze River resistome is broad-spectrum. The diverse ARG subtypes in this study may be attributed to the samples from multiple habitats and the large studied area. In addition, ARGs in six sampled habitats strongly adhere to the distance-decay relationship. This may be caused by the dispersal limitation of ARB/bacterial community [14,61] in the Yangtze River or the different sources of antibiotic resistance [55] in different regions of the Yangtze River.

4.2. Habitats-a bottleneck driving the dynamics of resistomes

ARGs exhibited different distribution patterns in the different sampled habitats. As reported in a previous study [60], the water habitats revealed more abundant and diverse ARGs than other riverine habitats. This suggests that water habitats may provide more ideal places for the growth and propagation of ARB. Certainly, it could also be that the water-borne bacteria were subjected to more selective pressure. We also found that the proportions of overlapped ARGs in the same Yangtze River habitat types were significantly higher than in other habitat pairs, suggesting that habitats are bottlenecks in driving ARG dynamics (ARG exchange) in riverine ecosystems. Our research emphasizes the importance of habitat differences in shaping resistance profiles, which enables a better understanding and prediction of antibiotic resistance evolution in the Yangtze River and elsewhere. A previous study has suggested that ARG transfers between soil bacteria are less likely than between human pathogens [62]. However, the mechanisms of ARG transfer between natural habitats are little known. Environmental variables, geographical separation, HGT, and ARB dispersal may be the drivers for the observed ARG coalescence between habitats of the Yangtze River. In the future, the mechanisms of ARG transfer between various habitats should be investigated in detail by combining experiments and genomic analyses.

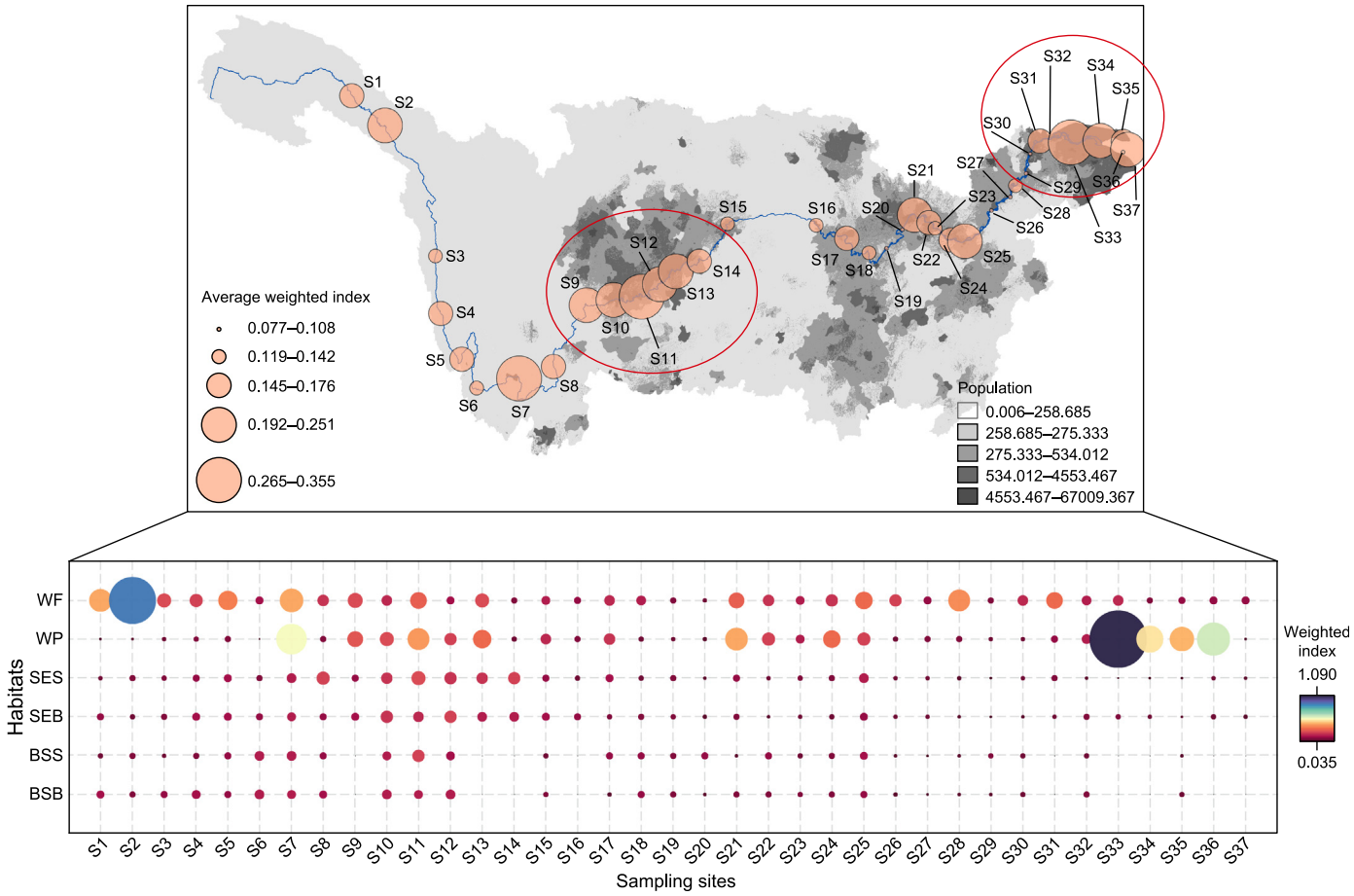

Fig. 4. Weighted index of ARG risk in the Yangtze River. All abbreviations are the same as in Fig. 1. The weighted index for each sample is shown in the figure below, and the average weighted index for each sampling site is depicted in the map above. Larger circles represent higher weighted indices for ARG risk.

4.3. Comparison of the ARG risk assessment methods

In contrast to the ARG risk assessment method developed in this study, other short sequence-based methods [21,52] would leave a large number of ARGs in the available databases unassessed for their health risk due to the limitations of the type and number of samples used. These will limit the use of these kinds of risk assessment methods in scientific research. Our assessment method provides a risk-ranking list of ARG sequences in the SARG database, which may greatly simplify the risk-ranking process in subsequent ARG studies. It is easy to provide researchers with a concise approach to assessing ARG risks in any sample. Short sequence-based methods may not capture the gene locations of ARGs compared to assembly-based methods, which may lead to errors in assessing the mobility and pathogenicity of ARGs. However, assembly-based risk assessment methods also have some limitations, such as not all reads getting assembled and errors occurring during assembly.

Although our study improves on current ARG risk assessment methods, some limitations remain, e.g., the ranking framework does not introduce phenotypic data, and bacterial hosts only include known pathogenic bacteria. Moreover, the current ARG risk assessment mainly relies on the available databases, greatly limiting our perspective on tackling the problem [21,23,52]. These limitations may still bias the obtained results and can only be overcome by the ongoing publication of newly available genomic data. Moreover, more complex factors affecting antibiotic resistance risk need to be considered, including various mutations leading to the evolution of new resistance determinants, natural selection amplifying the concentration of resistant strains, and the genetic context of HGT shuttling ARGs to pathogens [22]. Antibiotic resistance is spreading rapidly in natural and medical settings. The WHO's Global Antimicrobial Resistance Surveillance System allows global collaboration in investigating antibiotic resistance [21]. This action will facilitate the further development of ARG research and risk assessment.

4.4. Resistome risk priority regions of the Yangtze River

Our analysis indicates that the potential risks of ARGs in the two water-associated habitats were higher than in other habitats. As an important water source for coastal residents, agriculture, and ecological landscaping [63], the water security of the Yangtze River is closely related to public health. This emphasizes that ARGs in the water of the Yangtze River should be prioritized for health risk assessment. The significant positive correlations between ARG risks of particle-associated bacteria and anthropogenic activities highlight that ARG risks of particle-associated bacteria can serve as an important indicator of antibiotic resistance pollution arising from anthropogenic disturbance. As an important freshwater resource, the ARG risk of water-borne bacteria in rivers should be included as an important indicator for water quality assessments.

The weighted index of ARG risk in the Yangtze River revealed that the Chengdu–Chongqing urban and Yangtze River delta urban

agglomerations are the regions with higher ARG risk. These two regions should be the focus of future ARG risk prevention and control in the Yangtze River. Su et al. [64] analyzed the ARG burden based on the urban population in China's administrative districts, their results reveal that Sichuan and Jiangsu are the provinces along the Yangtze River with a high ARG abundance in the effluent of treatment plants, indicating that the resistome pollution in these two regions may be more serious than in other regions of the Yangtze River. This is confirmed by the results of this study.

As clearly stated in the "One Health" framework, humans live in an environment where their health is closely related to the environment and animal health. Environmental monitoring of ARGs is the first step toward making a real contribution to protecting ecosystems, animals, and human health. Only an in-depth analysis of antibiotic resistance in the river ecosystem can provide substantial data support for public health safety.

5. Conclusion

This study found that ARGs in the Yangtze River Basin are abundant and diverse, covering almost all antibiotic types. Compared with the sediments and bulk soils, resistome pollution in the water habitats of the Yangtze River indicated more severe contamination. Habitats exerted a bottleneck in ARG coalescence. ARG risk assessment in the Yangtze River discloses that ARG risks of particle-associated bacteria can indicate resistome pollution linked to anthropogenic disturbance. The Chengdu–Chongqing and the Yangtze River Delta urban agglomerations should be regarded as key regions for improving antibiotic resistance control. Our results provide an alternative perspective on ARG health risk assessment and have profound implications for maintaining the ecological and economic security of the Yangtze River basin for human water use.

CRediT authorship contribution statement

Chunxia Jiang: Conceptualization, Methodology, Investigation, Formal Analysis, Data Curation, Software, Visualization, Writing - Original Draft, Writing - Review & Editing. **Zelong Zhao:** Methodology, Data Curation, Software, Visualization, Writing - Review & Editing. **Hans-Peter Grossart:** Writing - Review & Editing. **Feng Ju:** Writing - Review & Editing. **Yi Zhao:** Writing - Review & Editing. **Geoffrey Michael Gadd:** Writing - Review & Editing. **Ewa Korzeniewska:** Writing - Review & Editing. **Yuyi Yang:** Conceptualization, Methodology, Investigation, Resources, Writing - Review & Editing, Supervision, Project Administration, Funding Acquisition.

Data availability

All data generated during this study is available at the Sequence Read Archive (SRA) under BioProject number PRJNA873262.

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.

Acknowledgments

This work was supported by the National Natural Science Foundation of China [Grant NO. 32071614 and U23A2059], the Starting Research Fund and Opening Research Fund from Key Laboratory of Aquatic Botany and Watershed Ecology, Chinese Academy of Sciences [Grant NO. Y9519802 and E0520202]. The authors thank Weihong Zhang for his help in sample collection, Seungdae Oh for revising the paper, and James M. Tiedje for his suggestion on the content of the paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ese.2024.100388.

References

[1] World Health Organization, Antimicrobial Resistance: Global Report on Surveillance, World Health Organization, 2014. https://www.who.int/publications/i/item/9789241564748.
[2] J. O'Neill, Tackling Drug-Resistant Infections Globally: Final Report and Recommendations, Government of the United Kingdom, 2016. https://amr-review.org/sites/default/files/160518_Final%20paper_with%20cover.pdf.
[3] O. Jonas, A. Irwin, F. Berthe, F. Le Gall, P. Marquez, Drug-resistant Infections: a Threat to Our Economic Future, vol. 2, World Bank Group, 2017 final report (English); HNP/Agriculture Global Antimicrobial Resistance Initiative Washington, D.C. http://documents.worldbank.org/curated/en/323311493396993758/final-report.
[4] C. Dang, Y. Xia, M. Zheng, T. Liu, W. Liu, Q. Chen, et al., Metagenomic insights into the profile of antibiotic resistomes in a large drinking water reservoir, Environ. Int. 136 (2020) 105449, https://doi.org/10.1016/j.envint.2019.105449.
[5] G.D. Wright, Antibiotic resistance in the environment: a link to the clinic? Curr. Opin. Microbiol. 13 (5) (2010) 589–594, https://doi.org/10.1016/j.mib.2010.08.005.
[6] C.F. Nnadozie, O.N. Odume, Freshwater environments as reservoirs of antibiotic resistant bacteria and their role in the dissemination of antibiotic resistance genes, Environ. Pollut. 254 (2019) 113067, https://doi.org/10.1016/j.envpol.2019.113067.
[7] D.M.M. Al Salah, G.N. Ngweme, A. Laffite, J.-P. Otamonga, C. Mulaji, J. Poté, Hospital wastewaters: a reservoir and source of clinically relevant bacteria and antibiotic resistant genes dissemination in urban river under tropical conditions, Ecotoxicol. Environ. Saf. 200 (2020) 110767, https://doi.org/10.1016/j.ecoenv.2020.110767.
[8] M. Amato, D. Dasí, A. González, M.A. Ferrús, M.Á. Castillo, Occurrence of antibiotic resistant bacteria and resistance genes in agricultural irrigation waters from Valencia city (Spain), Agric. Water Manag. 256 (2021) 107097, https://doi.org/10.1016/j.agwat.2021.107097.
[9] F. Ju, K. Beck, X. Yin, A. Maccagnan, C.S. McArdell, H.P. Singer, et al., Wastewater treatment plant resistomes are shaped by bacterial composition, genetic exchange, and upregulated expression in the effluent microbiomes, ISME J. 13 (2) (2019) 346–360, https://doi.org/10.1038/s41396-018-0277-8.
[10] E. Zankari, H. Hasman, S. Cosentino, M. Vestergaard, S. Rasmussen, O. Lund, et al., Identification of acquired antimicrobial resistance genes, J. Antimicrob. Chemother. 67 (11) (2012) 2640–2644, https://doi.org/10.1093/jac/dks261.
[11] Y. Wang, Z. Yu, P. Ding, J. Lu, L. Mao, L. Ngiam, et al., Antidepressants can induce mutation and enhance persistence toward multiple antibiotics, Proc. Natl. Acad. Sci. U.S.A. 120 (5) (2023) e2208344120, https://doi.org/10.1073/pnas.2208344120.
[12] Y. Wang, J. Lu, J. Engelstädter, S. Zhang, P. Ding, L. Mao, et al., Non-antibiotic pharmaceuticals enhance the transmission of exogenous antibiotic resistance genes through bacterial transformation, ISME J. 14 (8) (2020) 2179–2196, https://doi.org/10.1038/s41396-020-0679-2.
[13] R. Singh, A.P. Singh, S. Kumar, B.S. Giri, K.-H. Kim, Antibiotic resistance in major rivers in the world: a systematic review on occurrence, emergence, and management strategies, J. Clean. Prod. 234 (2019) 1484–1505, https://doi.org/10.1016/j.jclepro.2019.06.243.
[14] Y.-G. Zhu, Y. Zhao, B. Li, C.-L. Huang, S.-Y. Zhang, S. Yu, et al., Continental-scale pollution of estuaries with antibiotic resistance genes, Nat. Microbiol. 2 (4) (2017) 16270, https://doi.org/10.1038/nmicrobiol.2016.270.
[15] D. Zheng, G. Yin, M. Liu, L. Hou, Y. Yang, X. Liu, et al., Metagenomics highlights the impact of climate and human activities on antibiotic resistance genes in China's estuaries, Environ. Pollut. 301 (2022) 119015, https://doi.org/10.1016/j.envpol.2022.119015.
[16] H.S. Gweon, M.J. Bowes, H.L. Moorhouse, A.E. Oliver, M.J. Bailey, M.C. Acreman, et al., Contrasting community assembly processes structure lotic bacteria metacommunities along the river continuum, Environ. Microbiol. 23 (1) (2021) 484–498, https://doi.org/10.1111/1462-2920.15337.
[17] J. Wang, R. Pan, P. Dong, S. Liu, Q. Chen, A.G.L. Borthwick, et al., Supercarriers of antibiotic resistome in a world's large river, Microbiome 10 (1) (2022) 111, https://doi.org/10.1186/s40168-022-01294-z.
[18] H. Chen, Y. Li, W. Sun, L. Song, R. Zuo, Y. Teng, Characterization and source identification of antibiotic resistance genes in the sediments of an interconnected river-lake system, Environ. Int. 137 (2020) 105538, https://doi.org/10.1016/j.envint.2020.105538.
[19] Y. Zhang, C. Liu, H. Chen, J. Chen, J. Li, Y. Teng, Metagenomic insights into resistome coalescence in an urban sewage treatment plant-river system, Water Res. 224 (2022) 119061, https://doi.org/10.1016/j.watres.2022.119061.
[20] C.M. Manaia, Assessing the risk of antibiotic resistance transmission from the

environment to humans: non-direct proportionality between abundance and risk, Trends Microbiol. 25 (3) (2017) 173–181, https://doi.org/10.1016/j.tim.2016.11.014.
[21] Z. Zhang, Q. Zhang, T. Wang, N. Xu, T. Lu, W. Hong, et al., Assessment of global health risk of antibiotic resistance genes, Nat. Commun. 13 (1) (2022) 1553, https://doi.org/10.1038/s41467-022-29283-8.
[22] M. Oh, A. Pruden, C. Chen, L.S. Heath, K. Xia, L. Zhang, MetaCompare: a computational pipeline for prioritizing environmental resistome risk, FEMS Microbiol. Ecol. 94 (7) (2018) fiy079, https://doi.org/10.1093/femsec/fiy079.
[23] J.L. Martínez, T.M. Coque, F. Baquero, What is a resistance gene? Ranking risk in resistomes, Nat. Rev. Microbiol. 13 (2) (2015) 116–123, https://doi.org/10.1038/nrmicro3399.
[24] A.-N. Zhang, J.M. Gaston, C.L. Dai, S. Zhao, M. Poyet, M. Groussin, et al., An omics-based framework for assessing the health risk of antimicrobial resistance genes, Nat. Commun. 12 (1) (2021) 4765, https://doi.org/10.1038/s41467-021-25096-3.
[25] B.M. Satinsky, B.C. Crump, C.B. Smith, S. Sharma, B.L. Zielinski, M. Doherty, et al., Microspatial gene expression patterns in the amazon river plume, Proc. Natl. Acad. Sci. U.S.A. 111 (30) (2014) 11085–11090, https://doi.org/10.1073/pnas.1402782111.
[26] S. Bao, Chemical Analysis for Agricultural Soil (Chinese), China Agriculture Press, Beijing, 2000.
[27] H. Liu, Z. Xiong, X. Jiang, G. Liu, W. Liu, Heavy metal concentrations in riparian soils along the Han River, China: the importance of soil properties, topography and upland land use, Ecol. Eng. 97 (2016) 545–552, https://doi.org/10.1016/j.ecoleng.2016.10.060.
[28] X. Jiang, W. Liu, L. Yao, G. Liu, Y. Yang, The roles of environmental variation and spatial distance in explaining diversity and biogeography of soil denitrifying communities in remote Tibetan wetlands, FEMS Microbiol. Ecol. 96 (5) (2020), https://doi.org/10.1093/femsec/fiaa063 fiaa063.
[29] W. Zhang, M. Lei, Y. Li, P. Wang, C. Wang, Y. Gao, et al., Determination of vertical and horizontal assemblage drivers of bacterial community in a heavily polluted urban river, Water Res. 161 (2019) 98–107, https://doi.org/10.1016/j.watres.2019.05.107.
[30] Z. Ren, Y. Zeng, X.e. Fu, G. Zhang, L. Chen, J. Chen, et al., Modeling macrozooplankton and water quality relationships after wetland construction in the Wenyuhe River Basin, China, Ecol. Model. 252 (2013) 97–105, https://doi.org/10.1016/j.ecolmodel.2012.08.025.
[31] A.M. Bolger, M. Lohse, B. Usadel, Trimmomatic: a flexible trimmer for Illumina sequence data, Bioinformatics 30 (15) (2014) 2114–2120, https://doi.org/10.1093/bioinformatics/btu170.
[32] X. Yin, X.-T. Jiang, B. Chai, L. Li, Y. Yang, J.R. Cole, et al., ARGs-OAP v2.0 with an expanded SARG database and Hidden Markov Models for enhancement characterization and quantification of antibiotic resistance genes in environmental metagenomes, Bioinformatics 34 (13) (2018) 2263–2270, https://doi.org/10.1093/bioinformatics/bty053.
[33] K. Pärnänen, A. Karkman, J. Hultman, C. Lyra, J. Bengtsson-Palme, D.G.J. Larsson, et al., Maternal gut and breast milk microbiota affect infant gut antibiotic resistome and mobile genetic elements, Nat. Commun. 9 (1) (2018) 3891, https://doi.org/10.1038/s41467-018-06393-w.
[34] Y. Yang, B. Li, F. Ju, T. Zhang, Exploring variation of antibiotic resistance genes in activated sludge over a four-year period through a metagenomic approach, Environ. Sci. Technol. 47 (18) (2013) 10197–10205, https://doi.org/10.1021/es4017365.
[35] E. Kristiansson, J. Fick, A. Janzon, R. Grabic, C. Rutgersson, B. Weijdegård, et al., Pyrosequencing of antibiotic-contaminated river sediments reveals high levels of resistance and gene transfer elements, PLoS One 6 (2) (2011) e17038, https://doi.org/10.1371/journal.pone.0017038.
[36] B. Li, Y. Yang, L. Ma, F. Ju, F. Guo, J.M. Tiedje, et al., Metagenomic and network analysis reveal wide distribution and co-occurrence of environmental antibiotic resistance genes, ISME J. 9 (11) (2015) 2490–2502, https://doi.org/10.1038/ismej.2015.59.
[37] D.E. Wood, S.L. Salzberg, Kraken: ultrafast metagenomic sequence classification using exact alignments, Genome Biol. 15 (3) (2014) R46, https://doi.org/10.1186/gb-2014-15-3-r46.
[38] J. Lu, S.L. Salzberg, Ultrafast and accurate 16S rRNA microbial community analysis using Kraken 2, Microbiome 8 (1) (2020) 124, https://doi.org/10.1186/s40168-020-00900-2.
[39] D. Li, R. Luo, C.-M. Liu, C.-M. Leung, H.-F. Ting, K. Sadakane, et al., MEGAHIT v1.0: a fast and scalable metagenome assembler driven by advanced methodologies and community practices, Methods 102 (2016) 3–11, https://doi.org/10.1016/j.ymeth.2016.02.020.
[40] D.W.D. Kang, J. Froula, R. Egan, Z. Wang, MetaBAT, an efficient tool for accurately reconstructing single genomes from complex microbial communities, PeerJ 3 (2015) e1165, https://doi.org/10.7717/peerj.1165.
[41] D.H. Parks, M. Imelfort, C.T. Skennerton, P. Hugenholtz, G.W. Tyson, CheckM: assessing the quality of microbial genomes recovered from isolates, single cells, and metagenomes, Genome Res. 25 (7) (2015) 1043–1055, https://doi.org/10.1101/gr.186072.114.
[42] S. Nurk, D. Meleshko, A. Korobeynikov, P.A. Pevzner, metaSPAdes: a new versatile metagenomic assembler, Genome Res. 27 (5) (2017) 824–834, https://doi.org/10.1101/gr.213959.116.
[43] N. Segata, D. Börnigen, X.C. Morgan, C. Huttenhower, PhyloPhlAn is a new method for improved phylogenetic and taxonomic placement of microbes, Nat. Commun. 4 (1) (2013) 2304, https://doi.org/10.1038/ncomms3304.
[44] D. Hyatt, G.L. Chen, P.F. Locascio, M.L. Land, F.W. Larimer, L.J. Hauser, Prodigal: prokaryotic gene recognition and translation initiation site identification, BMC Bioinf. 11 (2010) 119, https://doi.org/10.1186/1471-2105-11-119.
[45] S.F. Altschul, W. Gish, W. Miller, E.W. Myers, D.J. Lipman, Basic local alignment search tool, J. Mol. Biol. 215 (3) (1990) 403–410, https://doi.org/10.1016/S0022-2836(05)80360-2.
[46] D.H. Parks, M. Chuvochina, D.W. Waite, C. Rinke, A. Skarshewski, P.-A. Chaumeil, et al., A standardized bacterial taxonomy based on genome phylogeny substantially revises the tree of life, Nat. Biotechnol. 36 (10) (2018) 996–1004, https://doi.org/10.1038/nbt.4229.
[47] N.L. Bray, H. Pimentel, P. Melsted, L. Pachter, Near-optimal probabilistic RNA-seq quantification, Nat. Biotechnol. 34 (5) (2016) 525–527, https://doi.org/10.1038/nbt.3519.
[48] A.R. Wattam, J.J. Davis, R. Assaf, S. Boisvert, T. Brettin, C. Bun, et al., Improvements to PATRIC, the all-bacterial bioinformatics database and analysis resource center, Nucleic Acids Res. 45 (D1) (2017) D535–D542, https://doi.org/10.1093/nar/gkw1017.
[49] D. Knights, J. Kuczynski, E.S. Charlson, J. Zaneveld, M.C. Mozer, R.G. Collman, et al., Bayesian community-wide culture-independent microbial source tracking, Nat. Methods 8 (9) (2011) 761–763, https://doi.org/10.1038/nmeth.1650.
[50] Y. Benjamini, Y. Hochberg, Controlling the false discovery rate: a practical and powerful approach to multiple testing, J. R. Stat. Soc. Ser. B-Stat. Methodol. 57 (1) (1995) 289–300, https://doi.org/10.1111/j.2517-6161.1995.tb02031.x.
[51] M. Bastian, S. Heymann, M. Jacomy Gephi, An open source software for exploring and manipulating networks, Proceedings of the International AAAI Conference on Web and Social Media 3 (1) (2009) 361–362, https://doi.org/10.1609/icwsm.v3i1.13937.
[52] Y. Gao, W. Zhang, Y. Li, Microbial community coalescence: does it matter in the Three Gorges Reservoir? Water Res. 205 (2021) 117638 https://doi.org/10.1016/j.watres.2021.117638.
[53] Y. Wang, H. Li, Y. Li, H. Guo, J. Zhou, T. Wang, Metagenomic analysis revealed sources, transmission, and health risk of antibiotic resistance genes in confluence of Fenhe, Weihe, and Yellow Rivers, Sci. Total Environ. 858 (2023) 159913, https://doi.org/10.1016/j.scitotenv.2022.159913.
[54] D.G.J. Larsson, C.-F. Flach, Antibiotic resistance in the environment, Nat. Rev. Microbiol. 20 (5) (2022) 257–269, https://doi.org/10.1038/s41579-021-00649-x.
[55] S. Reddy, K. Kaur, P. Barathe, V. Shriram, M. Govarthanan, V. Kumar, Antimicrobial resistance in urban river ecosystems, Microbiol. Res. 263 (2022) 127135, https://doi.org/10.1016/j.micres.2022.127135.
[56] HARTMANN Science Centre, Relevant pathogens from A–Z. https://www.bode-science-center.com/center/relevant-pathogens-from-a-z.html.
[57] T.J. Sharpton, An introduction to the analysis of shotgun metagenomic data, Front. Plant Sci. 5 (2014) 209, https://doi.org/10.3389/fpls.2014.00209.
[58] S. Liu, P.F. Wang, X. Wang, J. Chen, Ecological insights into the elevational biogeography of antibiotic resistance genes in a pristine river: metagenomic analysis along the Yarlung Tsangpo River on the Tibetan Plateau, Environ. Pollut. 286 (2021) 117101, https://doi.org/10.1016/j.envpol.2021.117101.
[59] W.J. Song, H.Y. Chen, N.A. Xue, S.Z. Wang, Y.Y. Yang, Metagenomic binning and assembled genome analysis revealed the distinct composition of resistome and mobilome in the Ili River, Ecotoxicol. Environ. Saf. 242 (2022) 113886, https://doi.org/10.1016/j.ecoenv.2022.113886.
[60] H.Y. Jiang, R.J. Zhou, M.D. Zhang, Z.N. Cheng, J. Li, G. Zhang, et al., Exploring the differences of antibiotic resistance genes profiles between river surface water and sediments using metagenomic approach, Ecotoxicol. Environ. Saf. 161 (2018) 64–69, https://doi.org/10.1016/j.ecoenv.2018.05.044.
[61] S. Liu, P.F. Wang, C. Wang, X. Wang, J. Chen, Anthropogenic disturbances on antibiotic resistome along the Yarlung Tsangpo River on the Tibetan Plateau: ecological dissemination mechanisms of antibiotic resistance genes to bacterial pathogens, Water Res. 202 (2021) 117447, https://doi.org/10.1016/j.watres.2021.117447.
[62] K.J. Forsberg, S. Patel, M.K. Gibson, C.L. Lauber, R. Knight, N. Fierer, et al., Bacterial phylogeny structures soil resistomes across habitats, Nature 509 (7502) (2014) 612–616, https://doi.org/10.1038/nature13377.
[63] Changjiang Water Resources Commission of the Ministry of Water Resources, the People's Republic of China, Water Resources Bulletin for the Yangtze River Basin and Southwest Rivers (In Chinese); Changjiang Water Resources Commission of the Ministry of Water Resources, the People's Republic of China, 2019. http://www.cjw.gov.cn/zwzc/zjgb/cjlyjxnzhszygb/202009/P020220411405437433148.pdf.
[64] J.Q. Su, X.L. An, B. Li, Q.L. Chen, M.R. Gillings, H. Chen, et al., Metagenomics of urban sewage identifies an extensively shared antibiotic resistome in China, Microbiome 5 (2017) 84, https://doi.org/10.1186/s40168-017-0298-y.