

UC Davis

UC Davis Previously Published Works

Title

Antibiotic resistance genes in an urban river as impacted by bacterial community and physicochemical parameters

Permalink

<https://escholarship.org/uc/item/48r9n2vj>

Journal

Environmental Science and Pollution Research, 24(30)

ISSN

0944-1344

Authors

Zhou, Zhen-Chao

Zheng, Ji

Wei, Yuan-Yuan

et al.

Publication Date

2017-10-01

DOI

10.1007/s11356-017-0032-0

Peer reviewed

Antibiotic resistance genes in an urban river as impacted by bacterial community and physicochemical parameters

Zhen-Chao Zhou¹ · Ji Zheng¹ · Yuan-Yuan Wei¹ · Tao Chen¹ · Randy A. Dahlgren² · Xu Shang³ · Hong Chen¹

Received: 16 June 2017 / Accepted: 24 August 2017
© Springer-Verlag GmbH Germany 2017

Abstract Antibiotic resistance genes (ARGs) in urban rivers are a serious public health concern in regions with poorly planned, rapid development. To gain insights into the predominant factors affecting the fate of ARGs in a highly polluted urban river in eastern China, a total of 285 ARGs, microbial communities, and 20 physicochemical parameters were analyzed for 17 sites. A total of 258 unique ARGs were detected using high-throughput qPCR, and the absolute abundance of total ARGs was positively correlated with total organic carbon and total dissolved nitrogen concentrations ($P < 0.01$). ARG abundance and diversity were greatly altered by microbial community structure. Variation partitioning analysis showed that the combined effects of multiple factors contributed to the profile and dissemination of ARGs, and variation of microbial communities was the major factor affecting the distribution of ARGs. The disparate distribution of some bacteria, including *Bacteroides* from mammalian gastrointestinal flora, *Burkholderia* from zoonotic infectious diseases, and *Zoogloea* from wastewater treatment, indicates that the urban

river was strongly influenced by point-source pollution. Results imply that microbial community shifts caused by changes in water quality may lead to the spread of ARGs, and point-source pollution in urban rivers requires greater attention to control the transfer of ARGs between environmental bacteria and pathogens.

Keywords Antibiotic resistance genes · Urban rivers · Physicochemical parameters · Microbial community structure · High-throughput qPCR

Introduction

Urban environments are vital for human health and sustainable development, and globally, more than 50% of the world population now live in urban areas (Statista 2016; Peña-Fernández et al. 2014). Urban rivers in rapidly developing regions are often severely impacted by uncontrolled population growth and lagging infrastructure development (Singh et al. 2010; Enger et al. 2012). As such, riverine pollution is a serious and growing public health challenge with antibiotic resistance genes (ARGs) regarded as a critical emerging contaminant (Pruden et al. 2006; Xu et al. 2016; Coutinho et al. 2014). Aquatic ecosystems are ideal environments for diverse ARGs (Amos et al. 2014; Nakayama et al. 2017) with high levels of ARGs detected in urban rivers (Ouyang et al. 2015; Xu et al. 2016; Rodriguez-Mozaz et al. 2015). Recently, Di Cesare et al. (2017) found the load of ARGs from rivers was correlated to rainfall, and thus runoff from surrounding lands. The occurrence and dissemination of ARGs in urban river systems have increasingly become a crucial issue in relation to water quality and human health because of the widespread and intensive use of surface waters (Angebault and Andreumont 2013; Levy and Marshall 2004; Martínez and Baquero 2014).

Responsible editor: Robert Duran

Electronic supplementary material The online version of this article (<https://doi.org/10.1007/s11356-017-0032-0>) contains supplementary material, which is available to authorized users.

✉ Hong Chen
chen_hong@zju.edu.cn

- ¹ Department of Environmental Engineering, College of Environmental and Resource Sciences, Zhejiang University, Hangzhou 310058, China
- ² Department of Land, Air and Water Resources, University of California, Davis, CA 95616, USA
- ³ Institute of Wenzhou Applied Technology in Environmental Research, Wenzhou Medical University, Wenzhou 325035, China

Czekalski et al. (2015) found that human activities strongly impact natural antibiotic resistance in the environment. However, the major factors contributing to the prevalence and fate of ARGs in urban rivers are largely unknown. Several factors, including microbial community structure, environmental parameters, geographic factors, and anthropogenic activities, have been proposed to shape ARG profiles (Chen et al. 2013; Forsberg et al. 2014; Cabral et al. 2016; Czekalski et al. 2015).

Recent studies have demonstrated that variations in microbial community structure and mobile genetic elements (MGEs) were significantly correlated with ARG profiles in soil, sludge, and drinking water (Forsberg et al. 2014; Jia et al. 2015; Su et al. 2015). However, riverine microbiota are exposed to a number of environmental factors including toxins, nutrients, hypoxia, and habitat alterations. Previous studies have examined only a limited number of physicochemical parameters and therefore do not provide enough information for an overall assessment of the distribution of microbiota and ARGs in urban river systems. Severe anthropogenic pollution in urban rivers can strongly affect aquatic environments and directly influence riverine microbiota (Jordaan and Bezuidenhout 2016; Zhang et al. 2015). Any shift in bacterial communities will further impact the nitrogen and carbon cycles and that in turn will affect microbiota (Hale et al. 2015; Ibekwe et al. 2016).

In this study, we examined 20 physicochemical parameters from 17 sites along the Wen-Rui Tang River watershed of eastern China. Due to rapid industrial growth and urbanization in recent decades, large volumes of wastewater from untreated sewage and service-oriented enterprises are discharged directly into the Wen-Rui Tang River (Mei et al. 2014). The total length of the river with tributaries is 1178 km, and the length of the main stem is 33.9 km (Mei et al. 2014). High-throughput quantitative PCR (HT-qPCR) with 296 primer sets (285 ARGs, 9 transposase genes, one class 1 integron-integrase gene, and the bacterial 16S ribosomal RNA (rRNA) gene) was used to detect microbial community composition and ARG profiles (Zhu et al. 2013).

The purpose of this study was to document the prevalence of ARGs and their relationship with bacterial communities and water quality parameters in the Wen-Rui Tang River. Microbial community structures, geographical factors, and physicochemical parameters were measured to assess the key drivers shaping the diversity and abundance of ARGs in the urban river system. Results of this study have broad relevance as the range of conditions examined in the Wen-Rui Tang River is representative of rivers in rapidly developing urban areas throughout the world.

Materials and methods

Sample collection and DNA extraction

Water samples were collected in June 2016 from the Wen-Rui Tang River in Wenzhou, China (Fig. 1a), and included five

main stem sites (A1, A2, A3, A4, and A5), seven first-branch tributaries (B1, B2, B3, B4, B5, B6, and B7), four second-branch tributaries (C1, C2, C3, and C4), and one suburban site (D1). The average depth of the sampled rivers was 2.4 m depending on sample sites. The 17 water samples were aseptically collected in triplicate from 10 to 20 cm below the water surface with sterile containers, refrigerated during transport to the lab, and analyzed within 24 h. Samples for DNA extraction were collected by vacuum filtration of water onto 0.22- μm pore-size nitrocellulose filters (Millipore, USA) until the filter clogged. The total water volume filtered was recorded for subsequent calculations. Three replicates were prepared for each site, and the membrane filters were carefully transferred to sterile silver paper specimen bags for storage at $-80\text{ }^{\circ}\text{C}$. DNA extraction of each filter was performed using a commercial kit (FastDNA SPIN Kit for soil, MP Biomedicals, Santa Ana, CA, USA) according to the manufacturer's instructions. In order to exclude heterogeneity of each sample and experimental bias, we randomized DNA extraction from the three replicate filters from each site. The purity and concentration of DNA extracts were determined by 1.5% agar gel electrophoresis and spectrophotometry (NanoDrop ND-2000c, Thermo, Waltham, MA USA).

Physicochemical analysis

Twenty physicochemical parameters were analyzed including temperature, pH, oxidation-reduction potential (ORP), transparency, turbidity, electrical conductivity (EC), dissolved oxygen (DO), suspended solids (SSs), chlorophyll-a (Chl-a), total nitrogen (TN), dissolved nitrogen (TDN), nitrate (NO_3^-), nitrite (NO_2^-), ammonium (NH_4^+), total phosphorus (TP), total dissolved phosphorus (TDP), phosphate (PO_4^{3-}), total carbon (TC), total organic carbon (TOC), and inorganic carbon (IC). All analysis followed standard methods (Mei et al. 2014).

HT-qPCR and data analysis

HT-qPCR of ARGs was performed using the SmartChip Real-time PCR (Warfengen Inc., Fremont, CA, USA). A total of 296 primer sets targeted 285 ARGs, 9 transposases, one clinical class 1 integron-integrase gene, and 16S rRNA genes (Zhu et al. 2013; Su et al. 2015). The 285 ARGs conferred resistance to almost all major antibiotics (aminoglycoside, beta-lactams, fluoroquinolone/quinolone/florfenicol/chloramphenicol/amphenicol (FCA), macrolide/lincosamide/streptogramin B (MLSB), sulfonamide, tetracycline, vancomycin, and genes coding multidrug efflux pumps or antibiotic deactivation protein resistance to other antibiotic and bactericide (other/efflux)) and covered three resistance mechanisms (antibiotic deactivation, efflux pumps, and cellular protection). All primers are listed in Table S1.

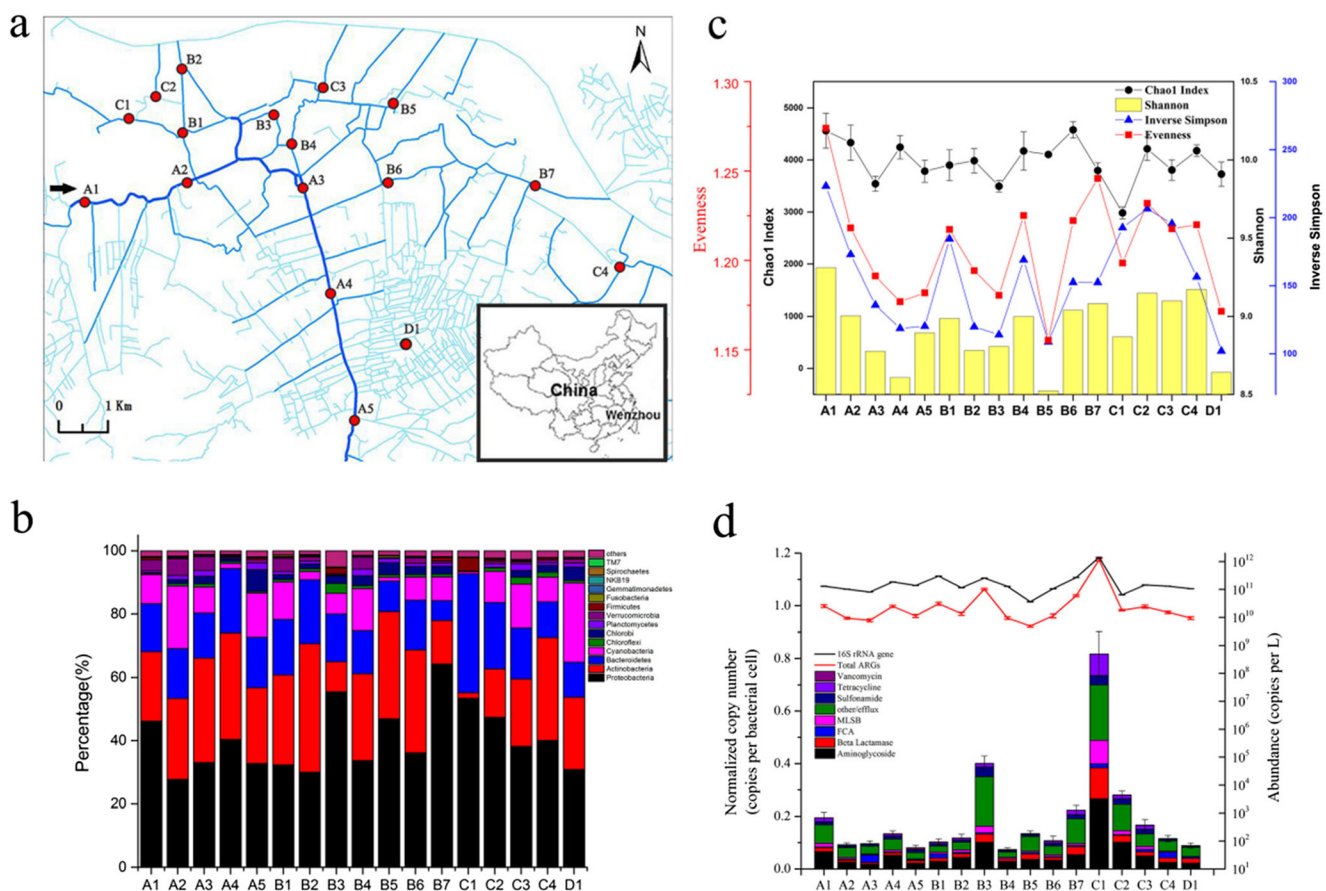


Fig. 1 Sampling points along the Wen-Rui Tang River watershed of eastern China (a). Average percentages of total 16S rRNA gene sequences classified to each phylum among the urban river samples (b).

Evenness, Chao1, Shannon, and inverse Simpson indices showing the diversity of bacterial communities in urban river samples (c). The normalized copy number of ARGs in different urban river samples (d)

The thermal cycle followed 10 min at 95 °C, 40 cycles of denaturation at 95 °C for 30 s, and annealing at 60 °C for 30 s. Finally, melting curve analyses were automatically generated by the program. For each primer set, amplification was conducted in triplicate and a non-template control was included; amplification efficiency outside the range of 1.8–2.2 was discarded. A comparative threshold cycle (C_T) method was used to determine ARGs' fold change (FC value) for water samples compared to the control (Schmittgen and Livak 2008). Relative gene copy number of ARGs = $10^{(31-C_T)}$ ($10/3$), where C_T refers to the quantitative PCR results and 31 refers to the detection limit (Zhu et al. 2017). Only samples with three repeats that had amplification were regarded as positive and used in further data analysis. The relative copy numbers of ARGs and MGEs generated by the HT-qPCR were transformed into absolute copy numbers by normalization to the absolute 16S rRNA gene copy numbers, which were determined by the standard curve (SC) quantification method.

Absolute 16S rRNA gene copy numbers were determined by SYBR Green I qPCR on a Bio-Rad iQ5 Real-Time PCR (Bio-Rad, Hercules, CA). The qPCR methods of determination of 16S rRNA abundance were described in detail by Chen

and Zhang (2013). All qPCRs were performed in technical triplicates with negative controls.

Bacterial 16S rRNA gene sequencing and analysis

To characterize the microbiota, the V4-V5 region of 16S rRNA genes was amplified using the universal forward 515F (5'-GTGCCAGCMGCCGCGG-3') and reverse 907R (5'-CCGTCAATTCMTTTRAGTTT-3') primers (Cui et al. 2015), and a 7-nucleotide barcode was added to the 5' end of each primer for identification of individual samples in a mixture within a single pyrosequencing run. Each 25 μL PCR contained 17.2 μL ddH₂O, 2.5 μL 10× PCR buffer, 2.5 mM dNTP mixture, 10 μL of each primer, 1.5 U of Taq DNA polymerase, and 1 μL of template DNA. The thermal cycle followed initial denaturation at 95 °C for 5 min, 24 cycles of denaturation at 95 °C for 30 s, annealing at 56 °C for 30 s, extension at 72 °C for 90 s, and a final extension at 72 °C for 8 min. The DNA bands were excised and purified followed by NanoDrop ND-2000c analysis to determine the quality and concentration of DNA. Quantitative Insights Into Microbial Ecology (QIIME) was used to process and analyze the high-

quality sequences. Sequences with more than 97% similarity were assigned to an operational taxonomic unit (OTU) by UCLUST (Edgar 2010).

Statistical analysis

Pearson correlation and one-way analysis of variance (ANOVA) were conducted using SPSS v20.0 (IBM, Armonk, NY, USA). A $P < 0.05$ value was considered statistically significant. Bar charts and line charts were generated by OriginPro 9.1 (OriginLab, Northampton, MA, USA). The partial Mantel test was used to determine correlations among environmental parameters and bacterial communities. Principal coordinate analysis (PCoA) was performed to evaluate differences in ARG profiles among samples based on the Bray–Curtis distance of ARG relative abundance. Mantel tests and redundancy analysis (RDA) were used to determine the correlation between ARG profiles and bacterial communities. Adonis test was conducted to determine significance differences in ARG profiles or bacterial community structure among samples. PCoA (Bray–Curtis distance) and RDA were conducted using Canoco software (v5.0). Shannon diversity, variation partitioning analysis (VPA), Adonis test, Mantel test, partial Mantel test, and heatmaps were prepared in R3.3.1 with vegan and pheatmap packages (Su et al. 2015).

Results

Physicochemical parameters

Sites showed a wide range in physical and chemical water quality properties as summarized in Table S2. Site C1 was unique among sites having the lowest DO and ORP, but the highest TN, TDN, TDP, PO_4^{3-} , NH_4^+ , TC, TOC, and IC concentrations ($P < 0.05$).

Characterization of bacterial community

A total of 531,019 reads with an average of 31,236 high-quality sequences per sample were obtained from all 17 samples. These sequences clustered into 6111 OTUs at a 3% dissimilarity level. Proteobacteria, Bacteroidetes, Actinobacteria, and Cyanobacteria were the dominant phyla in all samples, accounting for 86.5–96.0% of total bacterial 16S rRNA gene sequences (Fig. 1b). Urban rivers may receive untreated sewage and rainfall/runoff from surrounding lands that contain abundant nutrients. Some severely contaminated sites are known to experience periodic cyanobacterial blooms throughout the year. Betaproteobacteria was the dominant class in the phylum Proteobacteria (Fig. S1a). In the phylum Bacteroidetes, Saprospirae, Bacteroidia, and Flavobacteriia were the three dominant classes, and Bacteroidia showed

higher abundance at site C1 than other sites (Fig. S1b). The Actinobacteria phylum was primarily composed of the Actinobacteria class (Fig. S1c). Chloroplast was the dominant class contributing to variations in the Cyanobacteria phylum (Fig. S1d). At the genus level, *C39*, *Limnohabitans*, and *Polynucleobacter* within the Proteobacteria phylum were the three most dominant genus, accounting for 5.3–25.1% of total bacterial 16S rRNA gene sequences (Fig. 2). The relative abundance of bacterial genera, including *Bacteroides* and *Prevotalla* within the Bacteroidetes phylum, increased at site C1 (Fig. 2). Sites B3, B7, and C1 had higher abundances of *C39* and *Dechloromonas* than others, while *Synechococcus*, *Sediminibacterium*, and *Fluviicola* were lower at those sites (Fig. 2). Overall, these results indicate that geographical factors posed no obvious effect on microbial community structures. Furthermore, bacterial diversity decreased at sites A4, B5, and C1 (Fig. 1c), which was confirmed by evaluation of the Evenness, Chao1 estimator, and Shannon index.

PCoA based on the Bray–Curtis distance (Fig. 3a) revealed a significant shift (Adonis test, $P < 0.01$) of the overall microbial community composition. PCoA showed that microbial community structures were significantly different between water samples from site C1 and others. The first two PCs explained 81.0% of the total variance in microbial community structure, with PC1 explaining 60.0% of the variance. This demonstrates that the urban river samples could be grouped into three clusters: cluster A including A1, B3, B7, and C2; cluster B including A2, A3, A5, B1, B4, C4, and D1; and cluster C including A4, B2, B5, B6, and C3 (Fig. 3a). These results suggest that geographical factors were not the main factor contributing to the diversity and abundance of bacterial communities in the urban river system.

Diversity and abundance of ARGs

HT-qPCR was performed to investigate the abundance and diversity of ARGs. A total of 294 genes (285 ARGs, 8 transposase genes, the class 1 integron-integrase gene, and 16S rRNA genes) were detected in the water samples. The number of ARGs detected in each sample ranged from 100 to 159, with an average of 124 (Fig. S2). Site C1 (second-branch tributary) had the highest number of detected ARGs, followed by site B7 (first-branch tributary), and site A5 (main stem) with the lowest. The 258 unique ARGs were primarily grouped into eight types (aminoglycoside, beta-lactams, FCA, MLSB, other/efflux, sulfonamide, tetracycline, and vancomycin resistance genes), in which aminoglycoside, other/efflux, and beta-lactams resistance genes were most abundant (Fig. S2). The whole ARG profiles are shown in Fig. S3. Among the persistent ARGs in all samples, aminoglycoside, other/efflux, and beta-lactams resistance genes dominated (*aadA2-03*, *aadA1*, *aadA-02*, *aadA2-02*, *aadA-01*, *aacA_aphD*, *aacC2*, *qacEΔ1*, *qacH-02*, *bla_{GES}*, *bla_{OXA1}*–*bla_{OXA30}*,

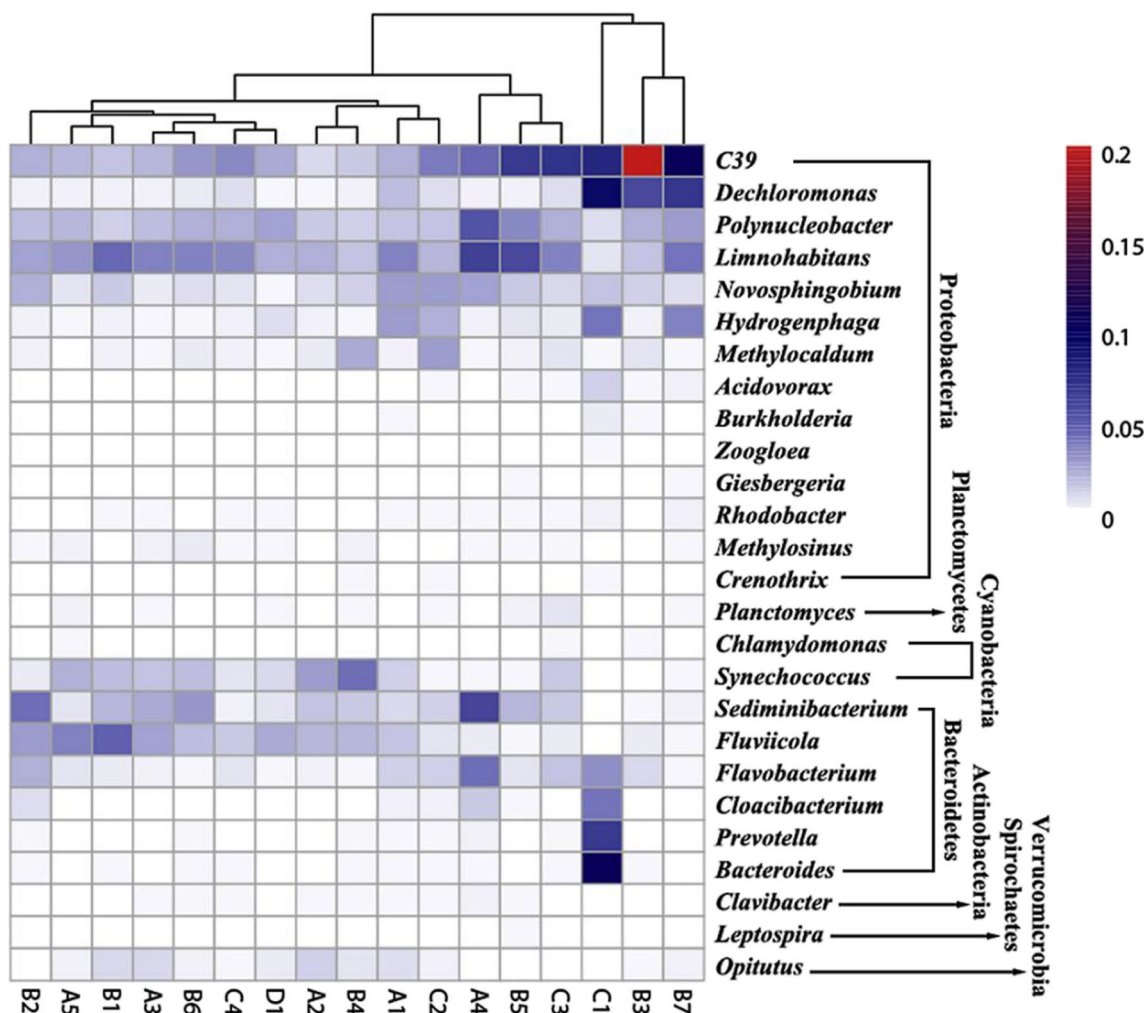


Fig. 2 Relative abundance of each taxonomic genus (>0.1% in any sample) in urban river samples. The color intensity in each panel shows the genus percentage in each sample

*bla*_{OXA10-01}, *bla*_{OXA10-02}, *bla*_{PER}, *bla*_{TEM}, *bla*_{VEB}, and *cfxA*) (Fig. S4). Generally, the absolute abundance of ARGs ranged from 3.61×10^{10} to 3.02×10^{11} copies/L in surface water

samples, which was higher than those found in Beijing urban rivers (Xu et al. 2016). The absolute abundance of detected ARGs and MGEs were significantly higher at site C1 than

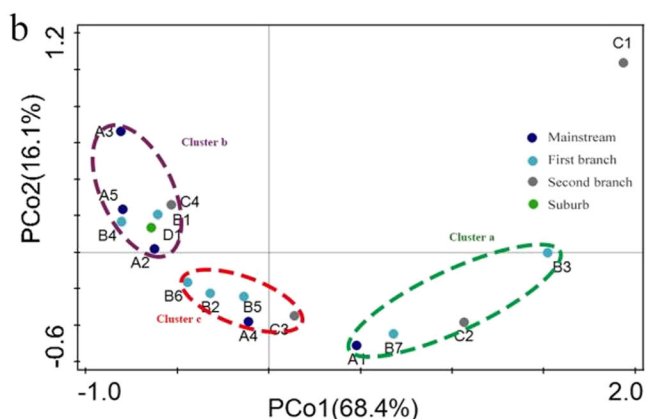
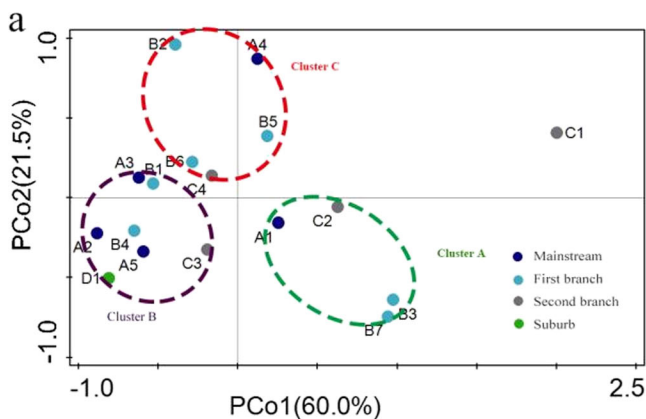


Fig. 3 Principal coordinate analysis (PCoA) based on Bray–Curtis distance showed the overall distribution pattern of **a** bacterial communities and **b** ARG profiles among urban river samples. Arrows represent the 14 genera (*Limnohabitans* (G1), *Fluviicola* (G2),

Synechococcus (G3), *Arcobacter* (G4), *Prevotella* (G5), *Acidovorax* (G6), *Bacteroides* (G7), *Paludibacter* (G8), *Hydrogenophaga* (G9), *Cloacibacterium* (G10), *Dechloromonas* (G11), *Thauera* (G12), *C39* (G13), and *Flavobacterium* (G14))

other sites (Fig. S5; $P < 0.05$), suggesting that the pollution and ARGs in the urban rivers were significantly different from the upstream waters (approximated by upstream site A1).

The normalized copy number of ARGs was used to assess the abundance of ARGs in the total bacterial community. The relative copy number of ARGs was similar among sites (range 0.07–0.82) with an average of 0.12 resistance genes per cell (Fig. 1d). The normalized copy number of ARGs increased at sites B3 and C1, which were more than two times greater than site A1. The relative copy number of the detectable ARGs was similar in main stem sites ($P > 0.05$). The relative copy number of detectable ARGs in site B3 was significantly higher than other samples of first-branch tributaries ($P < 0.05$). Furthermore, the relative copy number of detectable ARGs showed significant differences among the samples of sites C1, C2, C3, and C4 ($P < 0.05$), suggesting that the relative copy number of ARGs in tributaries was more sensitive than main stem sites.

ARGs detected in the urban water samples encompassed the three major resistance mechanisms: antibiotic deactivation (40%), efflux pumps (30%), and cellular protection (25%). Inverse Simpson, Shannon, and Evenness indices indicated that the diversity of detected ARGs at most sites was similar (Fig. S6). PCoA based on the Bray–Curtis distance showed that site C1 was distinct from the other sites (Fig. 3b). Furthermore, ARG profiles (Fig. 3b) in main stem sites (A1, A2, A3, A4, and A5), first-branch tributaries (B1, B2, B3, B4, B5, B6, and B7), second-branch tributaries (C1, C2, C3, and C4), and the suburban site (D1) showed random distributions, suggesting that geography was not a strong driver of ARG profile shifts in the urban river system. The first two PCs explained 84.5% of the total variance of ARGs, with PC1 explaining 68.4% of the variance (Fig. 3b). PCoA further demonstrated the spatial distribution characteristics based on the abundance of resistance genes in bacterial communities. The three ARG clusters in PCoA were similar to the PCoA of bacterial communities, which indicates that bacterial

communities were significantly correlated with ARGs in this urban river system.

Correlation between environmental factors, bacterial communities, and ARGs

Relationships among physicochemical parameters and bacterial communities were evaluated with RDA. Based on the PCoA analysis, the 17 sites were divided into three groups: group A (A1, B3, B7, and C2), group B (A2, A3, A5, B1, B4, C4, and D1), and group C (A4, B2, B5, B6, and C3). RDA and partial Mantel tests revealed that the bacterial communities were significantly correlated with TOC, TN, TDN, TDP, PO_4^{3-} , NH_4^+ , TC, and IC ($P < 0.01$ each; Fig. 4a). TOC, TN, TDN, TDP, PO_4^{3-} , NH_4^+ , TC, and IC were positively correlated with the first axis (explaining 38.5% of total variance), and TP was positively correlated with the second axis (explaining 10.2% of total variance). Pearson correlation analysis also indicated that the relative abundances of C39, *Dechloromonas*, *Limnohabitans*, *Acidovorax*, *Arcobacter*, *Cloacibacterium*, *Prevotella*, *Paludibacter*, and *Bacteroides* were significantly correlated with TOC, TN, TDN, TDP, PO_4^{3-} , NH_4^+ , TC, and IC ($P < 0.05$). The overall pattern of the bacterial community was significantly related to TN, TDN, TDP, and TOC (partial Mantel test; $P < 0.01$), suggesting that carbon energy sources and major nutrients (N&P) were key factors driving the microbial community composition in this urban river system. The abundance of total ARGs was positively correlated with 16S rRNA gene copy number (Fig. 1d; $P < 0.01$), which increased at sites B3 and C1.

RDA and Mantel tests showed that the ARG profiles were significantly correlated to bacterial composition and structure (Mantel test; $P < 0.001$), suggesting that the bacterial community significantly influenced the abundance and diversity of ARGs. RDA of bacterial communities and detected ARGs showed that sampling sites could be grouped into three clusters: cluster 1 including A1, B3, B7, and C2;

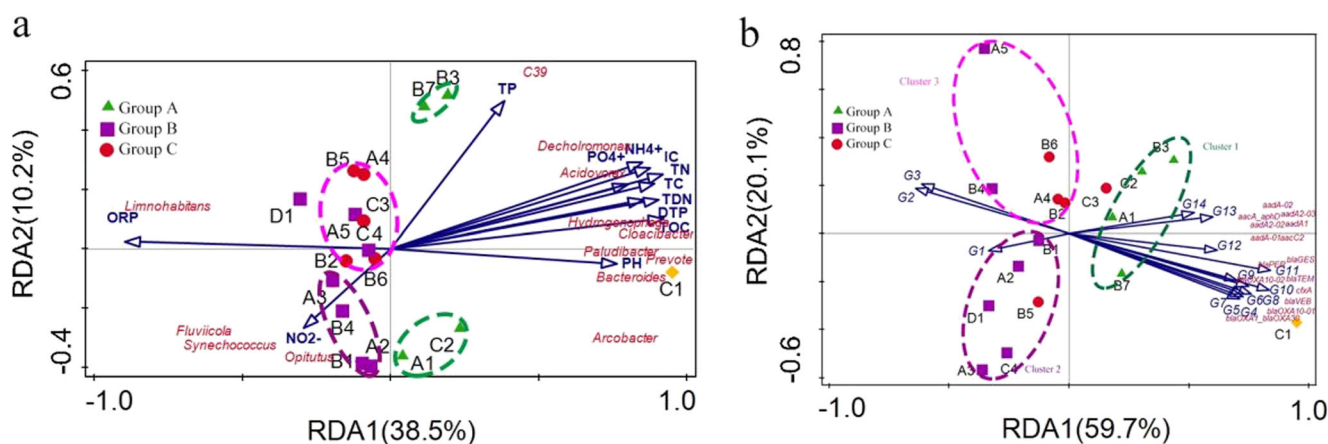


Fig. 4 Redundancy analysis (RDA) assessing the relationship between **a** physicochemical characteristics and bacterial communities and **b** bacterial communities and ARGs

including A4, A5, B2, B4, and B6; and cluster 3 including A2, A3, B1, B5, C4, and D1. This analysis reveals that bacterial communities greatly altered ARG abundance and diversity (Fig. 4b). Among the identified genera, 14 genera significantly correlated with persistent ARG distribution (*aadA2-03*, *aadA1*, *aadA-02*, *aadA2-02*, *aadA-01*, *aacA_aphD*, *aacC2*, *qacEΔ1*, *qacH-02*, *bla_{GES}*, *bla_{OXA1}*, *bla_{OXA30}*, *bla_{OXA10-01}*, *bla_{OXA10-02}*, *bla_{PER}*, *bla_{TEM}*, *bla_{VEB}*, and *cfxA*) in the urban river system ($P < 0.05$), including *Limnohabitans* (G1), *Fluviicola* (G2), *Synechococcus* (G3), *Arcobacter* (G4), *Prevotella* (G5), *Acidovorax* (G6), *Bacteroides* (G7), *Paludibacter* (G8), *Hydrogenophaga* (G9), *Cloacibacterium* (G10), *Dechloromonas* (G11), *Thauera* (G12), *C39* (G13), and *Flavobacterium* (G14). G4–G14 were positively correlated with the first axis (explaining 59.7% of total variance) and site C1, suggesting that G4–G14 played important roles in the dissemination and variation of ARGs. The relative abundances of *Arcobacter*, *Burkholderia*, *Dechloromonas*, *Hydrogenophaga*, *Acidovorax*, *Cloacibacterium*, *Prevotella*, *Paludibacter*, and *Bacteroides* were significantly correlated with aminoglycoside, beta-lactams, MLSB, sulfonamide, and tetracycline resistance genes, and the abundance of transposases (Table S3; $P < 0.05$). The identified genera within the Proteobacteria phylum (*Arcobacter*, *Hydrogenophaga*, and *Acidovorax*) and Bacteroidetes phylum (*Cloacibacterium*, *Prevotella*, *Paludibacter*, and *Bacteroides*) appear to affect the abundance and diversity of ARGs and mobile genetic elements.

Transposases, TOC, and TDN were positively correlated with the absolute abundance of total ARGs (Fig. 5a) and showed a stronger correlation with the abundance of aminoglycosides, beta-lactams, MLSB, other/efflux, and tetracycline resistance genes (Table 1; $P < 0.01$). The abundance of transposases was not significantly correlated with physicochemical parameters or bacterial communities ($P > 0.05$). These results suggest that the ARG profiles were affected by the combined effects of transposases, water quality, and microbial community composition. VPA demonstrated that over 80% of the observed variation in ARG profiles were explained, with microbial community composition shift contributing to 68.4% of the ARG variation (Fig. 5b). Thus, microbial community composition had a much stronger relationship with ARG variation than the physicochemical parameters and MGE alterations.

Discussion

This study systematically investigated the abundance and diversity of ARGs in an urban river system receiving considerable inputs of non-treated sewage and industrial waste. The abundance and diversity of ARGs in this urban river system were higher than previously reported from other Chinese

urban rivers (Luo et al. 2010; Xu et al. 2016; Ouyang et al. 2015). Among the genes found in all water samples, *aadA2-03*, *aadA1*, *bla_{VEB}*, *bla_{TEM}*, and *qacEΔ1* were consistently detected at high abundance. Previous studies showed high numbers of *bla_{TEM}* gene copies in surface water and sediments (Lachmayr et al. 2009; Singh et al. 2016; Bogaerts et al. 2016). The TEM-β-lactamases encoded by the *bla_{TEM}* gene represents one of the most clinically significant families of β-lactamases. The *qacEΔ1* genes represent a mechanism for antibiotic efflux resistance genes, which are commonly embedded in integron resistance cassettes (Singh et al. 2005; Koczura et al. 2016). The high level of *qacEΔ1* genes may result from the overuse and abuse of quaternary ammonium compounds which have widespread commercial applications (Ruan et al. 2014; Wassenar et al. 2015) that may lead to an increasing prevalence of resistance genes impacting human health. We also found high levels of vancomycin resistance genes which are relatively rare in natural environments with an absolute abundance range from 4.44×10^6 to 8.17×10^8 copies per L. Vancomycin is a fallback therapy against microorganisms in hospitals suggesting that wastewater from medical facilities could be a source of ARGs as inferred in previous studies (Zhu et al. 2017; D'Costa et al. 2011). The transposase gene was detected in each sample, indicating a potential for horizontal gene transfer of ARGs (Ouyang et al. 2015). The difference in bacterial community composition among the 17 water sample was demonstrated by 16S rRNA sequencing, and the ARG distribution revealed significant differences as well.

The urban river system contained high concentrations of several pollutants, with the high carbon, nitrogen, and phosphorous concentrations having obvious effects on the structure of bacterial communities and distribution of ARGs. However, there was no clear spatial distribution in the abundance and type of ARGs, indicating that ARG profiles were not significantly affected by geographical factors (Xu et al. 2016). Furthermore, we found that the abundance and diversity of ARGs from main stem sites were more stable than those from tributaries. The main stem sites had a low flow at the time of sampling and therefore received waters from similar upstream sources. In contrast, the tributaries are typically stagnant except following major rainfall events and are therefore affected by more local factors, such as contrasting bacterial communities and anthropogenic pollution sources.

The relative abundance of *Burkholderia* was higher at sites A1, B3, and C1 (Fig. 2), which contained some pathogenic members (e.g., *Burkholderia mallei* responsible for glanders disease that occurs in horses and related animals, *Burkholderia pseudomallei* a causative agent of melioidosis, *Burkholderia cepacia* an important pathogen for pulmonary infections in humans with cystic fibrosis) (Woods and Sokol 2006; Djordjevic et al. 2013). These findings are consistent with site A1 being the furthest upstream site that is closest to areas with animal husbandry and sites B3 and C1 being in the

Fig. 5 Correlation of total absolute abundance of ARGs with transposases, TOC, and TDN (a). Variation partitioning analysis (VPA) differentiating effects of bacterial community, physicochemical characteristics, and mobile genetic elements (MGEs) on ARG profiles in river samples (b)

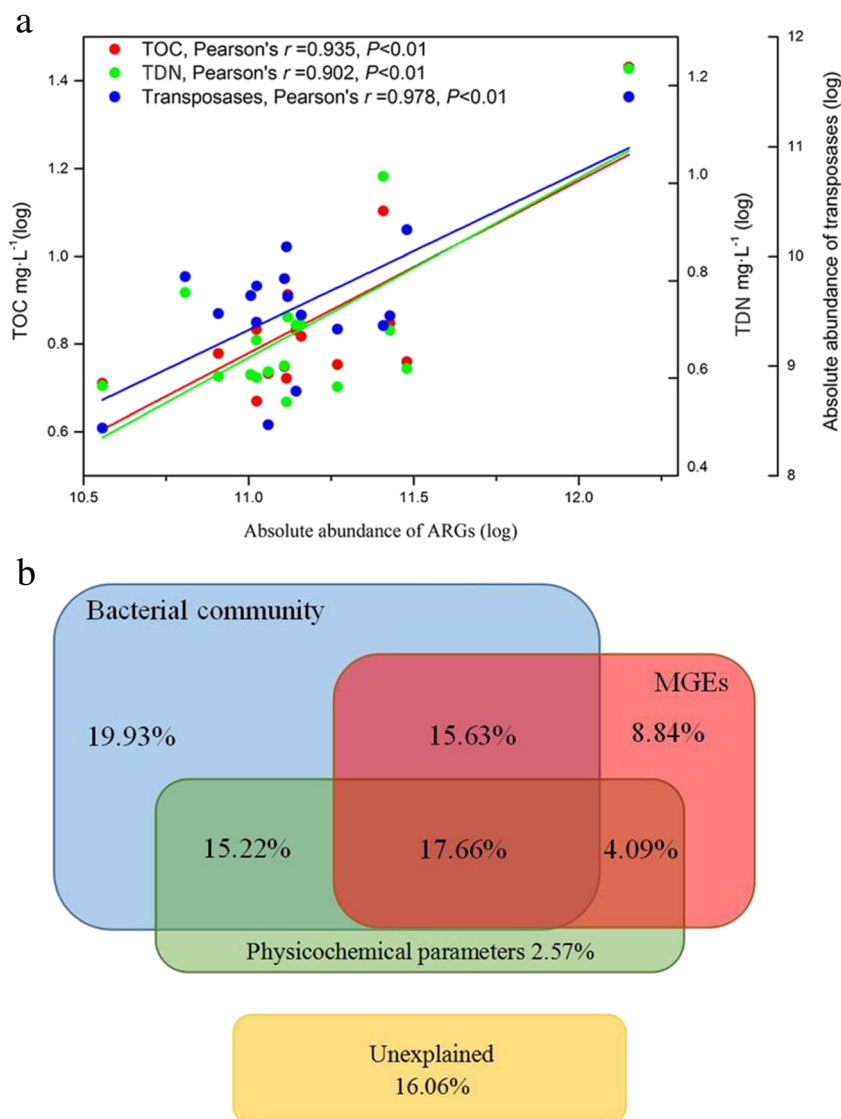


Table 1 Pearson correlations among transposases, TOC, TDN, and ARGs

	Abundance of transposases	TOC	TDN
Aminoglycoside	0.802 ^a	0.945 ^a	0.930 ^a
Beta-lactams	0.761 ^a	0.967 ^a	0.939 ^a
FCA	0.199	0.247	0.193
MLSB	0.805 ^a	0.964 ^a	0.947 ^a
Other/efflux	0.550 ^b	0.851 ^a	0.887 ^a
Sulfonamide	0.474	0.777 ^a	0.836 ^a
Tetracycline	0.812 ^a	0.931 ^a	0.898 ^a
Vancomycin	-0.251	-0.081	-0.040
Total ARGs	0.744 ^a	0.962 ^a	0.959 ^a

^a Correlation is significant at the 0.01 level (two-tailed)

^b Correlation is significant at the 0.05 level (two-tailed)

urban environment and subject to receiving waters from hospital waste and non-treated sewage. The relative abundance of *Burkholderia* was significantly correlated with ARGs (aminoglycoside, beta-lactams, MLSB, sulfonamide, and tetracycline resistance genes) and transposases (Table S3; $P < 0.01$), demonstrating that MGEs and ARGs were prevalent in zoonotic bacteria. Among all sites, site C1 had the highest abundance of several bacteria (e.g., *Zoogloea*, *Bacteroides*, and *Prevotalla*), and *Zoogloea* species that commonly exist in wastewater and play a crucial role in organismal remediation in wastewater treatment (Garrity et al. 2005; Shao et al. 2009). This suggests that site C1 may be receiving large inputs of sewage waste, consistent with the extremely high NH_4^+ concentration (16.4 mg $\text{NH}_4\text{-N/L}$) at this site. Furthermore, *Bacteroides* species are obligate anaerobic bacteria, playing an important role in the mammalian gastrointestinal flora and making up a significant portion of the fecal bacterial population (Wexler 2007; Madigan et al. 2012).

Thus, its higher abundance may be associated with sewage inputs which contribute to the low DO and high TOC and TDN concentrations at C1. The high inputs of anthropogenic contaminants at C1 (likely from non-treated sewage inputs) appear to amplify the proportion of opportunistic bacteria and shift the composition of the bacterial community.

Environmental factors, such as TOC, TN, TDN, TDP, PO_4^{3-} , NH_4^+ , TC, and IC, are key factors driving bacterial community shifts in urban river systems (Jordaan and Bezuidenhout 2016). Our data identified TOC and TDN as potentially important environmental factors affecting the abundance and diversity of bacterial communities and ARGs in urban river systems as based on RDA analysis. However, the abundance of transposases was only significantly correlated with the ARGs. Moreover, VPA demonstrated that bacterial community variation was more strongly associated with ARGs than MGEs (Jordaan and Bezuidenhout 2016; Zhao et al. 2016). We found that 14 genera were positively correlated with the ARG distribution, indicating that bacterial communities shaped the distribution and abundance of ARGs (Huerta et al. 2013; Xiong et al. 2015). Since environmental factors can greatly affect the microbial community composition, they would be expected to cause variation of ARG profiles in the urban river environment (Su et al. 2015). Environmental factors and bacteria communities may directly and indirectly affect the variation of ARGs, suggesting that the combined effects of multiple, interacting factors contribute to the profile and dissemination of ARGs in urban river systems.

Anthropogenic activity significantly alters the water and habitat quality of urban rivers causing a shift in microbial community composition (Fu et al. 2014; Koczura et al. 2016; Lu et al. 2015; Zhu et al. 2017). The distribution of ARGs exhibited no significant spatial heterogeneity, indicating that the point-source pollution released into the urban river system is widespread and has a strong influence on riverine microbiota and ARG profiles.

Conclusions

This study concludes that ARG profiles were modified by the combined effects of water quality, MGEs, bacterial community structure, and anthropogenic activity. In particular, the concentrations of carbon resources, nitrogen, and phosphorous were strongly correlated with variations in bacterial community structure. TOC and TDN were the primary water quality factors contributing to variations in ARG profiles. Changes in microbial communities caused by variations in physicochemical parameters of river waters was the major factor shaping ARG profiles rather than geographical factors and MGEs in the urban river system. Point-source pollution in urban rivers may be an important factor affecting the widespread abundance of ARGs.

Acknowledgements This work was supported by the Natural Science Foundation of China (21677121 and 41571130064).

References

- Amos GCA, Zhang L, Hawkey PM, Gaze WH, Wellington EM (2014) Functional metagenomic analysis reveals rivers are a reservoir for diverse antibiotic resistance genes. *Vet Microbiol* 171(3–4):441–447
- Angebault C, Andremont A (2013) Antimicrobial agent exposure and the emergence and spread of resistant microorganisms: issues associated with study design. *Eur J Clin Microbiol* 32(5):581–595
- Bogaerts P, Cuzon G, Evrard S, Hoebekeet M, Naas T, Glupczynski Y (2016) Evaluation of a DNA microarray for rapid detection of the most prevalent extended-spectrum β -lactamases, plasmid-mediated cephalosporinases and carbapenemases in *Enterobacteriaceae*, *Pseudomonas* and *Acinetobacter*. *Int J Antimicrob Agents* 48(2): 189–193
- Cabral L, Júnior GVL, Pereira-de-Sousa ST, Dias CF, Lira-Cadete L, Andreote FD, Hess M (2016) Anthropogenic impact on mangrove sediments triggers differential responses in the heavy metals and antibiotic resistomes of microbial communities. *Environ Pollut* 216:460–469
- Chen H, Zhang M (2013) Effects of advanced treatment systems on the removal of antibiotic resistance genes in wastewater treatment plants from Hangzhou, China. *Environ Sci Technol* 47:8157–8163
- Chen B, Liang X, Huang X, Zhang T, Li X (2013) Differentiating anthropogenic impacts on ARGs in the Pearl River Estuary by using suitable gene indicators. *Water Res* 47(8):2811–2820
- Coutinho FH, Silveira CB, Pinto LH, Salloto GRB, Cardoso AM, Martins OB, Clementino MM (2014) Antibiotic resistance is widespread in urban aquatic environments of Rio de Janeiro, Brazil. *Microb Ecol* 68(3):441–452
- Cui E, Ying W, Zuo Y, Hong C (2015) Effect of different biochars on antibiotic resistance genes and bacterial community during chicken manure composting. *Bioresour Technol* 203:11–17
- Czekalski N, Sigdel R, Birtel J, Matthews B, Bürgmann H (2015) Does human activity impact the natural antibiotic resistance background? Abundance of antibiotic resistance genes in 21 Swiss lakes. *Environ Int* 81:45–55
- D'Costa VM, King CE, Kalan L, Morar M, Sung WW, Schwarz C, Debruyne R (2011) Antibiotic resistance is ancient. *Nature* 477(7365):457–461
- Di Cesare A, Eckert EM, Rogora M, Corno G (2017) Rainfall increases the abundance of antibiotic resistance genes within a riverine microbial community. *Environ Pollut* 226:473–478
- Djordjevic SP, Stokes HW, Chowdhury PR (2013) Mobile elements, zoonotic pathogens and commensal bacteria: conduits for the delivery of resistance genes into humans, production animals and soil microbiota. *Front Microbiol* 4(86):1–12
- Edgar RC (2010) Search and clustering orders of magnitude faster than BLAST. *Bioinformatics* 26(19):2460–2461
- Enger KS, Nelson KL, Clasen T, Roseet JB, Eisenberg JNS (2012) Linking quantitative microbial risk assessment and epidemiological data: informing safe drinking water trials in developing countries. *Environ Sci Technol* 46(9):5160–5167
- Forsberg KJ, Patel S, Gibson MK, Lauber CL, Knight R, Fierer N, Dantas G (2014) Bacterial phylogeny structures soil resistomes across habitats. *Nature* 509(7502):612–616
- Fu Y, Tang C, Li J, Zhao Y, Wei Z, Zeng X (2014) Sources and transport of organic carbon from the Dongjiang River to the Humen outlet of the Pearl River, southern China. *J Geogr Sci* 24(1):143–158

- Garrity G, Brenner DJ, Kreig N, (2005) *Bergey's manual of systematic bacteriology*, Vol. 2 Part C. The Alpha-, Beta-, Delta-, and Epsilonproteobacteria. Springer, New York
- Hale RL, Turnbull L, Earl SR, Childers DL, Grimm NB (2015) Stormwater infrastructure controls runoff and dissolved material export from arid urban watersheds. *Ecosystems* 18(1):62–75
- Huerta B, Marti E, Gros M, Armengol J (2013) Exploring the links between antibiotic occurrence, antibiotic resistance, and bacterial communities in water supply reservoirs. *Sci Total Environ* 456–457(7):161–170
- Ibekwe AM, Ma J, Murinda SE (2016) Bacterial community composition and structure in an urban river impacted by different pollutant sources. *Sci Total Environ* 566–567:1176–1185
- Jia S, Shi P, Hu Q, Li B, Zhang T, Zhang XX (2015) Bacterial community shift drives antibiotic resistance promotion during drinking water chlorination. *Environ Sci Technol* 49(20):12271–12279
- Jordaan K, Bezuidenhout CC (2016) Bacterial community composition of an urban river in the North West Province, South Africa, in relation to physico-chemical water quality. *Environ Sci Pollut Res* 23(6):5868–5880
- Koczura R, Mokracka J, Taraszewska A, Łopacinska N (2016) Abundance of class 1 integron-integrase and sulfonamide resistance genes in river water and sediment is affected by anthropogenic pressure and environmental factors. *Microb Ecol* 72(4):1–8
- Lachmayr KL, Kerkhof LJ, Dirienzo AG, Cavanaugh CM, Ford TE (2009) Quantifying nonspecific TEM β -lactamase (blaTEM) genes in a wastewater stream. *Appl Environ Microb* 75(1):203–211
- Levy SB, Marshall B (2004) Antibacterial resistance worldwide: causes, challenges and responses. *Nat Med* 10(12):122–129
- Lu Z, Na G, Gao H, Wang L, Bao C, Yao Z (2015) Fate of sulfonamide resistance genes in estuary environment and effect of anthropogenic activities. *Sci Total Environ* 527–528:429–438
- Luo Y, Mao D, Rysz M, Zhou Q, Zhang H, Xu L, Alvarez PJJ (2010) Trends in antibiotic resistance genes occurrence in the Haihe River, China. *Environ Sci Technol* 44(19):7220–7225
- Madigan MT, Martinko JM, Stahl D, Clark D (2012) *Brock biology of microorganisms*, 13th edn. Pearson/Benjamin Cummings, San Francisco, pp 368–381
- Martínez JL, Baquero F (2014) Emergence and spread of antibiotic resistance: setting a parameter space. *Upsala J Med Sci* 119(2):68–77
- Mei K, Liao L, Zhu Y, Lu P, Wang Z, Dahlgren RA, Zhang M (2014) Evaluation of spatial-temporal variations and trends in surface water quality across a rural-suburban-urban interface. *Environ Sci Pollut Res* 21(13):8036–8051
- Nakayama T, Tuyet-Hoa TT, Harada K, Warisaya M, Asayama M, Hinenoya A, Lee WJ, Phu TM, Ueda S, Sumimura Y, Hirata K, Phuong NT, Yamamoto Y (2017) Water metagenomic analysis reveals low bacterial diversity and the presence of antimicrobial residues and resistance genes in a river containing wastewater from backyard aquacultures in the Mekong Delta, Vietnam. *Environ Pollut* 222:294–306
- Ouyang WY, Huang FY, Zhao Y LH, Su JQ (2015) Increased levels of antibiotic resistance in urban stream of Jiulongjiang River, China. *Appl Microbiol Biotechnol* 99(13):5697–5707
- Peña-Fernández A, González-Muñoz MJ, Lobo-Bedmar MC (2014) Establishing the importance of human health risk assessment for metals and metalloids in urban environments. *Environ Int* 72(22):176–185
- Pruden A, Pei R, Storteboom H, Carlson KH (2006) Antibiotic resistance genes as emerging contaminants: studies in northern Colorado. *Environ Sci Technol* 40(23):7445–7450
- Rodriguez-Mozaz S, Chamorro S, Marti E, Martia E, Huertaa B, Grosa M, Sánchez-Melsiúa A, Borrego CM, Barcelóa D, Balcázar JL (2015) Occurrence of antibiotics and antibiotic resistance genes in hospital and urban wastewaters and their impact on the receiving river. *Water Res* 69:234–242
- Ruan T, Song S, Wang T, Liu R, Lin Y, Jiang G (2014) Identification and composition of emerging quaternary ammonium compounds in municipal sewage sludge in China. *Environ Sci Technol* 48(8):4289–4297
- Schmittgen TD, Livak KJ (2008) Analyzing real-time PCR data by the comparative C(T) method. *Nat Protoc* 3(6):1101–1108
- Shao Y, Chung BS, Lee SS, Park W, Jeon CO (2009) *Zoogloea caeni* sp. nov. a floc-forming bacterium isolated from activated sludge. *Int J Syst Evol Microbiol* 59(3):526–530
- Singh R, Schroeder CM, Meng J, White DG, McDermott PF, Wagner DD, Walker RD (2005) Identification of antimicrobial resistance and class 1 integrons in Shiga toxin-producing *Escherichia coli* recovered from humans and food animals. *J Antimicrob Chemother* 56(1):216–219
- Singh G, Vajpayee P, Ram S, Shanker R (2010) Environmental reservoirs for enterotoxigenic *Escherichia coli* in South Asian Gangetic riverine system. *Environ Sci Technol* 44(16):6475–6480
- Singh G, Vajpayee P, Rani N, Amoah ID, Stenström TA, Shanker R (2016) Exploring the potential reservoirs of non specific TEM beta lactamase (blaTEM) gene in the Indo-Gangetic region: a risk assessment approach to predict health hazards. *J Hazard Mater* 314:121–128
- Statista (2016) Degree of urbanization by continent in 2016. <http://www.statista.com/statistics/270860/urbanization-by-continent/>
- Su JQ, Wei B, Ouyang WY, Huang FY, Zhao Y, Xu HJ, Zhu YG (2015) Antibiotic resistance and its association with bacterial communities during sewage sludge composting. *Environ Sci Technol* 49(12):7356–7363
- Wassenaar TM, Ussery D, Nielsen LN, Ingmer H (2015) Review and phylogenetic analysis of qac genes that reduce susceptibility to quaternary ammonium compounds in *Staphylococcus* species. *Eur J Immunol* 5(1):44–61
- Wexler HM (2007) Bacteroides: the good, the bad, and the nitty-gritty. *Clin Microbiol Rev* 20(4):593–621
- Woods DE, Sokol PA (2006) The genus *Burkholderia*. Springer, New York, pp 848–860
- Xiong W, Sun Y, Ding X, Wang M, Zeng Z (2015) Selective pressure of antibiotics on ARGs and bacterial communities in manure-polluted freshwater-sediment microcosms. *Front Microbiol* 6:1–8
- Xu Y, Guo C, Luo Y, Lv J, Zhang Y, Lin H, Xu J (2016) Occurrence and distribution of antibiotics, antibiotic resistance genes in the urban rivers in Beijing, China. *Environ Pollut* 213:833–840
- Zhang WS, Swaney DP, Li XY, Hong B, Howarth RW, Ding SH (2015) Anthropogenic point-source and non-point-source nitrogen inputs into Huai River Basin and their impacts on riverine ammonia-nitrogen flux. *Biogeosciences* 12:4275–4289
- Zhao Z, Wang J, Han Y, Chen J, Liu GF, Lu H, Yan B, Chen S (2016) Nutrients, heavy metals and microbial communities co-driven distribution of antibiotic resistance genes in adjacent environment of mariculture. *Environ Pollut* 220:909–918
- Zhu YG, Johnson TA, Su JQ, Qiao M, Guo GX, Stedtfeld RD, Hashsham SA, Tiedje JM (2013) Diverse and abundant antibiotic resistance genes in Chinese swine farms. *Proc Natl Acad Sci U S A* 110(9):3435–3440
- Zhu YG, Zhao Y, Li B, Huang CL, Zhang SY, Yu S, Chen YS, Zhang T, Gillings MR, Su JQ (2017) Continental-scale pollution of estuaries with antibiotic resistance genes. *Nature Microbiol* 2:1–7