Tracking antibiotic resistome during wastewater treatment using high throughput quantitative PCR

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Abstract

Wastewater treatment plants (WWTPs) contain diverse antibiotic resistance genes (ARGs), and thus are considered as a major pathway for the dissemination of these genes into the environments. However, comprehensive evaluations of ARGs dynamic during wastewater treatment process lack extensive investigations on a broad spectrum of ARGs. Here, we investigated the dynamics of ARGs and bacterial community structures in 114 samples from eleven Chinese WWTPs using high-throughput quantitative PCR and 16S rRNA-based Illumina sequencing analysis. Significant shift of ARGs profiles was observed and wastewater treatment process could significantly reduce the abundance and diversity of ARGs, with the removal of ARGs concentration by 1–2 orders of magnitude. Whereas, a considerable number of ARGs were detected and enriched in effluents compared with influents. In particular, seven ARGs mainly conferring resistance to beta-lactams and aminoglycosides and three mobile genetic elements persisted in all WWTPs samples after wastewater treatment. ARGs profiles varied with wastewater treatment processes, seasons and regions. This study tracked the footprint of ARGs during wastewater treatment process, which would support the assessment on the spread of ARGs from WWTPs and provide data for identifying management options to improve ARG mitigation in WWTPs.

1. Introduction

Antibiotic resistant pathogens have posed serious threat to human health worldwide owing to the increasing infection and mortality as well as treatment cost (Andersson and Hughes, 2010). Municipal wastewater treatment plants (WWTPs) are significant reservoirs for releasing antibiotic resistant bacteria and resistance determinants into environments (Chen et al., 2016; LaPara et al., 2011; Negreanu et al., 2012). WWTPs receive sewages from various sources (e.g. hospitals, industries and households) (Mao et al., 2015; Pruden et al., 2013), of which human commensal microorganisms are the main bacterial inputs to WWTPs. A proportion of human commensal microorganisms are resistant to antibiotics and thus resistant bacteria and resistance genes might arise in WWTPs. WWTPs are also considered as hotspots for horizontal gene transfer (HGT), enabling the development and dissemination of ARGs between bacteria (Karkman et al., 2016; Rizzo et al., 2013). High density of bacteria from different sources can interact and exchange resistance genes via HGT. In addition, chemical compounds (e.g. antibiotics and heavy metals) with varying concentrations can provide persistent pressure for selecting resistant bacteria, facilitating emergence of novel resistance determinants (LaPara et al., 2011; Novo and Manaia, 2010). Discharge of treated wastewater and sludge would lead to release of ARGs to downstream environments (Szczepanowski et al., 2009; LaPara et al., 2011; Caucci and Berendonk, 2014). In this regard, WWTPs link human activities and the environment, contributing to the occurrence, spread and persistence of...
resistant bacteria and ARGs in the environments (Chen et al., 2016; LaPara et al., 2011; Negreanu et al., 2012).

Previous efforts in investigating the occurrence and abundance of resistance genes in WWTPs indicated the removal efficiencies varied for different ARGs. Although most of studies have reported wastewater treatment can efficiently reduce the abundance of resistance genes (Diehl and LaPara, 2016; Novo et al., 2013; Zhang et al., 2009), there are reports showing that the effect of wastewater treatment on the relative abundance of ARGs is little and some resistance genes can even be enriched in effluent (Mao et al., 2015; Yang et al., 2014). This discrepancy may be partially due to the limited number of targeted ARGs. Profiling the dynamics of extensive number of ARGs during wastewater treatment process is critical for a comprehensive evaluation on the removal of resistant determinants. Metagenomic analysis and high-throughput qPCR array have been adopted to characterize ARGs in a limited number of WWTP(s), showing that the relative abundance of resistance genes varies with seasons and antibiotics in WWTPs show no direct selection for resistance genes (Bengtsson-Palme et al., 2016; Karkman et al., 2016; Yang et al., 2014). WWTPs receive wastewater from various sources daily with diverse bacterial communities and chemical components (Caucci et al., 2016; Sahoo et al., 2010), and thus the resistant bacteria loads during wastewater treatment might also change accordingly. However, the spatial and temporal distributions of resistome and bacterial community during the complete wastewater treatment process were not yet well addressed, especially at a large scale sampling level.

In this study, we collected 114 samples including influent, activated sludge and effluent from eleven WWTPs in six Chinese coastal cities. Illumina sequencing of bacterial 16S rRNA gene and high-throughput quantitative PCR (HT-qPCR) were applied (An et al., 2018) to provide an overview of the abundance and the composition of antibiotic resistance genes during wastewater treatment process; and (Andersson and Hughes, 2010) to characterize the seasonal and geographical distributions of resistome and bacterial community. By examining eleven Chinese WWTPs, we expect to obtain an in-depth understanding of the resistome during wastewater treatment process, and to provide data for mitigation of ARGs in WWTPs.

2. Materials and methods

2.1. Sample collection and DNA extraction

A total of 114 samples including influent (INF), activated sludge and effluent from eleven WWTPs in six Chinese coastal cities, Longyan, Xiamen, Nanjing, Shenzhen and Hong Kong in August 2014 (summer) and February 2014 (winter), respectively (Fig. S1 and Table S1). These WWTPs mainly treat municipal wastewater and use A/O (anaerobic/anoxic/aerobic) and oxidation ditch processes, in which two Hong Kong WWTPs (HK_ST and HK_SW) have considerable sources of saline (1.1% salinity) sewage and slaughterhouse wastewater, respectively (Zhang et al., 2012). Two parallel samples from each plant at the same season were taken within two consecutive days without rains to exclude the effect of the weather. When collecting the effluent samples, effects of hydraulic retention time were considered. Influent and effluent samples were collected in plastic containers (disinfected using 75% alcohol), and activated sludge was kept in sterilized polypropylene centrifuge tubes. All samples were mixed on site with an equal volume of 100% ethanol for biomass fixation. Fixed samples were kept on ice, and immediately delivered to the laboratory. To collect the bacterial pellet, each fixed influent sample (400 mL) was centrifuged at 10,000g for 20 min at 4 °C and fixed effluent samples (400 mL) were filtered through a 0.22 μm cellulose nitrate membrane. Fixed activated sludge sample (2 mL) was pelleted by centrifuging at 10,000g for 20 min at 4 °C. All concentrated samples were stored at −20 °C before DNA extraction (Li et al., 2015).

DNA was extracted using a FastDNA® Spin kit for Soil (MP Biomedicals, France) according to the manufacturer’s instructions with a modified step of 2 × 1 min bead beating for cellular disruption. DNA concentration and quality were assessed using a NanoDrop spectrophotometer (ND-1000, Nanodrop, USA).

2.2. HT-qPCR and data analysis

The relative quantification of ARGs (copies/16S rRNA gene) was evaluated by using the WaferGen Smart-Chip Real-Time PCR system (WaferGen Inc. USA) as described previously (Su et al., 2015). A total of 296 primer sets were used (Table S2), which targeted 285 ARGs concerning resistance to major classes of antibiotics, one 16S rRNA gene (Zhu et al., 2013), eight transposases, one class 1 integron-integrase gene (intI1) (Stokes et al., 2006) and one clinical class 1 integron-integrase gene (cintI1) (Gillings et al., 2015), respectively. Clinical class 1 integrons are recent descendants of a single evolution event involving in intI1 variants in environmental bacteria (Gillings et al., 2015). The two integron integrase genes and the eight transposase genes were classified into mobile genetic elements (MGEs) related to horizontal gene transfer. Amplification was conducted in a 100 nL reaction system and the thermal cycle was set according to the previous descriptions (Ou-yang et al., 2015; Wang et al., 2014). All qPCR reactions were conducted in triplicate and three negative controls without DNA template were included. qPCR conditions were set as following: initial enzyme activation at 95 °C for 10 min and 40 cycles of 95 °C 30 s, 60 °C 30 s. Melting curve analysis was conducted after amplification.

HT-qPCR data was analyzed using SmartChip qPCR software (V 2.7.0.1). Reactions with multiple peaks and reactions with amplification efficiency outside the acceptable range (90%–110%) were discarded (Ou-yang et al., 2015; Su et al., 2015; Wang et al., 2014). A threshold cycle (Ct) 31 was used as the cutoff value and positive samples should have three positive technical replicates. Relative copy numbers (copies/copy of 16S rRNA gene) of ARGs were calculated according to the previous study (Eq. (1)) (Loof et al., 2012). Fold change (FC) values of ARGs were calculated using a comparative Ct method to exhibit ARGs enrichment compared to influent samples (Schmittgen and Livak, 2008), based on the relative copy number of ARGs (copies/copy of 16S rRNA gene) using Excel 2010 (Microsoft Office 2010, Microsoft, USA) (Eq. (2)).

$$
\text{Gene copy number} = 10^{[(\text{Ct}_\text{ARG}) - \text{Ct}_{\text{16S}}]/10} \\
\Delta C_T = \text{Ct}_{\text{ARG}} - \text{Ct}_{\text{16S}} \\
\Delta C_T = \text{Ct}_{\text{ARG}} - \text{Ct}_{\text{16S}} \\
\text{FC} = 2^{-\Delta \Delta C_T} \\
\text{fold change} \text{(FC)} = \frac{\text{ARG in sample}}{\text{ARG in reference sample}} \\
\text{where } C_t \text{ is the threshold cycle, ARG is one of the 295 resistance genes, 16S refers to 16S rRNA gene, Target is the activated sludge or effluent samples and Ref refers to influent samples. Resistance genes are considered statistically enriched or decreased when } 2^{-\Delta \Delta C_T} > 1 \text{ or } < 1 (S \text{ is the standard deviation of the } \Delta \Delta C_T \text{ value), respectively.}
$$

2.3. Absolute quantification of 16S rRNA gene

The absolute copy number of 16S rRNA gene was quantified using a SYBR® Green approach. The qPCR (20 μL) was performed using a LightCycler Roche 480 (Roche Inc., USA) with a reaction system consisting of 10 μL SYBR® Premix Ex Taq™ II (2 ×) (Takara, Japan), 0.4 μM of each primer and 20 ng of DNA. PCR amplification was conducted under the following conditions: initial denaturation at 95 °C 3 min, 40 cycles of 95 °C 15 s, 60 °C 1 min and 72 °C 15 s. qPCR amplification of the standards was achieved using 10-fold serially diluted 16S rRNA gene incorporated plasmids. All qPCR assays were conducted in triplicate with negative and positive controls. Equal volume of E. coli DNA and sterile water were used as DNA templates in positive and negative
controls, respectively. Thus, the absolute concentration (copies/L) of 16S rRNA gene was calculated according to the volume of the sample used for DNA extraction.

A significant linear correlation between the abundance data of 16S rRNA gene from the SmartChip Real-Time System and the Roche 480 (r² = 0.81, P < 0.01) was observed. Thus, relative copy number of ARGs (copies/copy of 16S rRNA gene) retrieved from SmartChip Real-Time System could be transformed to ARG concentration using the formula: ARG concentration (copies/L) = (relative copy number of ARGs, copies/copy of 16S rRNA gene) × (concentration of 16S rRNA gene, copies/L) (Ou-yang et al., 2015).

2.4. Illumina sequencing and bioinformatics analysis

Among the 114 samples, 16S rRNA gene sequencing data (clean data) of 34 influent samples were downloaded from the National Center for Biotechnology Information (NCBI) Sequence Read Archive (ID SRP139793) (Su et al., 2017). For the remaining samples (activated sludge and effluent), Illumina sequencing of bacterial community was conducted according to our previous study (Su et al., 2015). Briefly, bacterial genomic DNA was amplified with a set of primers (515F and 907R) targeting the hypervariable V4-V5 region of the 16S rRNA gene (Turner et al., 1999). After purification and quantification, the resulting PCR products were pooled and sequenced on an Illumina MiSeq PE300 sequencing platform at Novogene Bioinformatics Technology Co. Ltd., Beijing, China.

The generated sequence reads were split using the barcodes and were analyzed using Quantitative Insights into Microbial Ecology (QIIME, v1.9.1) with the default parameters for each step (Caporaso et al., 2010a). Briefly, sequences were quality trimmed and low-quality reads were discarded if they contained ambiguous nucleotides, primer mismatches, homopolymers in excess of six bases, or errors in barcodes. Then primer regions were removed, sequences were filtered and denoised by Denoiser (Reeder and Knight, 2010), and chimera checking was performed using ChimeraSlayer (Quince et al., 2011). Sequences were clustered into operational taxonomic units (OTUs) at a minimum pair-wise identity of 97% with Uclust (Edgar, 2010). Singleton OTUs (number of sequences ≤2) were discarded to generate the final OTU table for subsequent analysis. The most abundant sequence from each OTU was selected as representative sequence, which were assigned to a taxonomy using a Ribosomal Database Project (RDP) classifier with a confidence threshold of 0.80 (80%) (Version 2.2) (Wang et al., 2007). Representative OTUs were aligned using a PyNAST aligner (Caporaso et al., 2010b), and a phylogenetic tree was then built using the FastTree algorithm (Price et al., 2010).

Rarefaction analysis was performed using QIIME by randomly extracting 20,169 clean sequences from each sample. OTU richness, PD_whole_tree phylogenetic diversity, Shannon diversity and Simpson index were calculated. For β-diversity analysis, the differences in overall community composition were measured and estimated by computing weighted UniFrac, unweighted UniFrac and Bray-Curtis distances between each pair of samples. All sequences were deposited in the NCBI Sequence Read Archive under the accession number SRP136450.

2.5. Statistical analysis

Redundancy analysis (RDA), non-metric multidimensional scaling (NMDS), variation partitioning analysis (VPA), Adonis test, cluster analysis and heatmap plotting were performed in R 3.2.3 (R Foundation for Statistical Computing; Vienna, Austria, 2014) with vegan 2.0–10 (Oksanen et al., 2007) and pheatmap package (Kolde, 2013). The hierarchical clustering analysis was conducted using the unweighted-pair group mean average (UPGMA) and between-groups linkage methods, based on the Bray-Curtis distance. One-Way analysis of variation (ANOVA), t-test and significance test were performed using SPSS V20.0 (IBM, USA) with statistical tests considered significant at P < 0.05. The P value was adjusted using FDR method to reduce the false-positive results (Benjamini and Hochberg, 1995). All possible pairwise Spearman’s rank correlations between ARGs and bacterial taxa were calculated and a correlation matrix was constructed in an R environment using “psych” package. A valid co-occurrence event was considered to be a robust correlation if the Spearman’s correlation coefficient (ρ) was > 0.7 and the P-value < 0.01 (Li et al., 2015). Gephi 0.9.1 software was applied for network visualization (Bastian et al., 2009).

3. Results

3.1. Diversity and abundance of ARGs in WWTPs

In total, 211 ± 43 ARGs and 5 ± 1 MGEs (including integrase genes and transposase genes) were detected in all samples, where influent samples harbored 118 ± 41 ARGs, activated sludge had 95 ± 46 ARGs and effluent contained 105 ± 50 ARGs (Fig. S2a). Aminoglycoside, multidrug, tetracycline and beta-lactam resistance genes were the most abundant ARG types during treatment process, accounting for 68% to 97% of the total ARG concentrations (Fig. S3). Wastewater treatment significantly reduced the abundance of ARGs with the removal efficiency of 1–2 orders of magnitude (P < 0.05,
antibiotics, including macrolide-lincosamide-streptogramin B (MLSB), beta-lactams, vancomycin, aminoglycosides and multidrug (Table S3). The maximum enrichment of 9533-fold for blaSHV-02 was detected in effluent. Seven ARGs and three MGEs were detected in all 114 samples, which accounted for 54.1%, 58.6% and 58.5% of the total concentrations (copy numbers) of detected ARGs in effluent, activated sludge and effluent, respectively (Fig. 4). The persistent ARGs included genes conferring resistance to aminoglycoside (aadA, aadA1 and aadA2), beta-lactams (blaVEM and blaOXA-10), MLSB (ereA) and multidrug (qacEdelta1) and MGEs encoding genes (IS613, trpA and clntI-1).

3.2. Seasonal and geographical variations of resistome in WWTPs

Winter samples harbored significantly higher ARG concentrations ($P < 0.05$) except in effluents (Fig. S4a), while relative abundance of ARGs showed no distinct variation between seasons (Fig. S4b). Summer had higher number of ARGs ($P > 0.05$) (Fig. S4c). Significant seasonal difference in the diversity of ARGs was observed in activated sludge ($P < 0.01$) (Fig. S4d). In addition, ARG distribution revealed significant seasonal differences (Adonis test, $P < 0.05$) and distinct groups were generated according to sampling time (Fig. 2b).

ARG copy number showed no distinct regional difference (Fig. S5a), while significantly higher relative abundance of ARGs was observed in WWTPs from mainland China ($P < 0.05$) (Fig. S5b). The detected number of ARGs in mainland was higher than that in Hong Kong ($P > 0.05$) (Fig. S5c), and mainland harbored more diverse ARGs than Hong Kong ($P < 0.01$) except in effluent (Fig. S5d). Significant geographical distribution of ARG profiles were observed with samples from mainland and Hong Kong were clustered together, respectively, and were well separated from each other (Adonis test, $P < 0.01$) (Fig. 2c and Fig. 5).

3.3. Characterization of bacterial community

A total of 5,788,455 high-quality sequences were generated from all 114 samples, ranging from 20,169 to 148,457 sequences per sample (median = 43,755), which were clustered into 73,138 OTUs at a threshold of 97% sequence similarity (average 5228 OTUs). Significant difference of bacterial diversity was not observed during wastewater treatment processes (Fig. S6a). In activated sludge samples, we observed a remarkable variation of OTU number with seasons ($P < 0.01$) (Fig. S6b). Additionally, OTU numbers in influent and activated sludge significantly varied with between mainland China and Hong Kong ($P < 0.05$), with higher bacterial richness in mainland WWTPs (Fig. S6c). Wastewater treatment process significantly altered the overall bacterial community structures as samples were clustered together according to the different treatment processes (Adonis test, $P < 0.01$) (Fig. 6a). Seasonal (Adonis test, $P < 0.01$) and geographical (Adonis test, $P < 0.01$) patterns of bacterial community were also observed in WWTPs (Fig. 6b and Fig. 6c). Similar to ARG profile, Hong Kong samples were separated from mainland WWTPs at both OTU and genus levels (Fig. S7).

The compositions of bacterial community displayed obvious variations with wastewater processes, seasons and regions at the phylum level (Fig. S8). Proteobacteria, Bacteroidetes, Firmicutes and Chloroflexi were the most dominant phyla, accounting for 81.2% of the total bacterial 16S rRNA gene sequences. The relative abundance of Bacteroidetes and Firmicutes decreased, while Proteobacteria increased after wastewater treatment (Fig. S8a). Bacterial composition in winter and summer was generally similar, except that winter effluents harbored more Bacteroidetes and Actinobacteria (Fig. S8b). Higher proportion of Chloroflexi and Chlorobi was detected in mainland samples (Fig. S8c).

3.4. Relationship between resistome and bacterial community

Network analysis was used to investigate co-occurrence patterns...
Fig. 3. Enrichment and attenuation of antibiotic resistance genes in activated sludge and effluent compared with influent. Fold change (FC) values are calculated based on the relative quantification data by the approach of ΔΔCt comparison, with influent samples as reference samples. The legend denotes log scale fold change values. Each row represents for a single primer set and rows were clustered based on Bray-Curtis distance. Numbers on the top of each column indicate the numbers of significantly enriched (red) or reduced (black) ARGs. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)
between ARGs and bacterial taxa (genus level). We hypothesized that the correlation analysis indicated the potential host information of ARGs when the ARGs exerted a strong and significantly positive correlation with co-occurred bacterial taxa ($r > 0.7$, adjusted $P > 0.01$). Our results revealed that three bacterial phyla, including Firmicutes, Actinobacteria and Bacteriodes, were the possible hosts of ARGs (Fig. S9 and Table S4). To explore the contribution of various factors to resistome profile, we employed VPA analysis to separate the effects of bacterial community and MGEs on the variation of ARGs basing on the relative abundances of ARGs and bacterial phyla (Fig. S10). A total of 59.8% of the ARGs variation was explained by bacterial community and MGEs, in which, shift in bacterial communities contributed to 16.7% of the total variation of ARGs.

### 4. Discussion

By using high throughput qPCR, we quantified antibiotic resistance genes in samples from eleven full-scale Chinese WWTPs using 295 sets of primers. To our best knowledge, this study provided by far the most comprehensive picture for ARG profiles in WWTPs. Seasonal and geographical distribution of ARGs profiles were observed in WWTP samples. We detected high abundance and diversity of ARGs and MGEs, some of which were persistent and enriched after wastewater treatment, potentially posing a risk of dissemination of resistance genes to the environments.

Wastewater treatment process significantly reduced the overall richness, concentration ($P < 0.05$) and relative abundance of ARGs ($P < 0.01$). This analysis was consistent with previous studies (Bengtsson-Palme et al., 2016; Karkman et al., 2016; Yang et al., 2014), indicating that wastewater treatment process could efficiently remove the bacteria carrying resistance genes in wastewater. The highest ARG richness were detected in influent samples, which was expected since urban sewage received a large amount of human commensal bacteria that carrying resistance genes (Bengtsson-Palme et al., 2016; Bouki et al., 2013; Yang et al., 2013). Activated sludge was found to harbor the highest ARGs concentrations, while the relative abundance and richness of ARGs were the lowest. These data indicated that the increase of ARG concentration in activated sludge might be, at least in part, due to the proliferation of total bacterial population, given that the bacterial concentration was the highest in activated sludge. Wastewater treatment could only partially remove ARGs and the reduction of ARG concentration might be largely driven by the decrease of total bacterial biomass (Bengtsson-Palme et al., 2016).

Wastewater treatment significantly changed the ARG profiles and different treatment processes harbored distinct resistance gene compositions. Previous studies showed that oxygen exposure, primary clarification process and activated sludge treatment might contribute to the variation of ARG profile during wastewater treatment (Bengtsson-Palme et al., 2016; Ravazzini et al., 2005; Voshol and Sak, 1968). Although significant removal of ARGs was observed after wastewater treatment process, a fairly high concentration of ARGs (averaging $4.8 \times 10^{10}$ copies/L) were detected in effluent, demonstrating that discharge of effluent could release resistance genes into environments.

A large proportion of resistance genes were also observed to be significantly enriched in activated sludge and effluent. As the last line against gram-positive resistant bacteria such as Clostridium difficile, vancomycin has been prudently used in human therapies and is restricted in animal industry, whereas enrichment of many vancomycin resistance genes in effluent was still observed in this study. A possible explanation was that avoparcin, one of the glycopeptide antibiotics, was widely used as feed additive for the growth promotion of animals (Wegener, 2003; Witte, 1998).

Seven shared ARGs and three MGEs were detected in all WWTP samples, highlighting the persistence of these genetic elements during wastewater treatment. These shared ARGs might enter into various environmental compartments by land application of sludge or effluent discharge. Aminoglycoside (aadA, aadA1 and aadA2) and beta-lactam (blaVEM and blaOXA-10) resistance genes were frequently detected ARGs in WWTPs, which were closely related to antibiotics important for human infections treatment or veterinary usage (Li et al., 2015; Zhu et al., 2013). For example, blaOXA-16 confers resistance to carbapenems (a critically important last-resort antibiotics class) was detected in all samples. Additionally, the detection of int-1 and qacEdelta1 in all samples and the significant correlation between the int-1, aminoglycosides and beta-lactams resistance genes (data not shown) indicated that the integron mediated horizontal gene transfer may significantly contribute to the persistence of these genes. This was supported by our recent observations that aminoglycosides and beta-lactams resistance genes were prevalent in integron gene cassettes (An et al., 2018).

Seasonal distributions of resistome and bacterial community were observed in WWTPs, indicating that the composition of bacterial community could be one of the major determinants of ARG content, affecting the distribution and the occurrence of ARGs in environments (Forsberg et al., 2014). The seasonal difference in the consumption of antibiotics in China (peaked in winter) (Van Boeckel et al., 2014) might also contribute to the seasonal variation of ARG profiles in WWTPs (Su et al., 2015). Geographical grouping of resistome and bacterial community in WWTPs was observed and the samples from mainland China were separated from Hong Kong samples. The difference in the sources of sewage between Hong Kong WWTPs (Shatin, ST; Shekwhui, SWH) and mainland China WWTPs might explain the variation of resistome profile. Shatin plant treats saline sewage (due to sea water toilet flushing practice) and Shekwhui treats sewage that contains a large proportion of slaughterhouse wastewater (Zhang et al., 2012).
5. Conclusions

This study presented comprehensive evidences that wastewater treatment could significantly reduce the richness and abundance of ARGs. However, a substantial number of ARGs were detected in wastewater effluents, which could be consequently discharged into downstream environments. Aminoglycoside and beta-lactams resistance genes persisted in all WWTP samples and were closely associated with integron 1 integrase, suggesting the need for monitoring and mitigating these genes in WWTPs.

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Notes

The authors declare no competing financial interest.

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