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Prevalence of antibiotic resistance genes in drinking water and biofilms: The correlation with the microbial community and opportunistic pathogens

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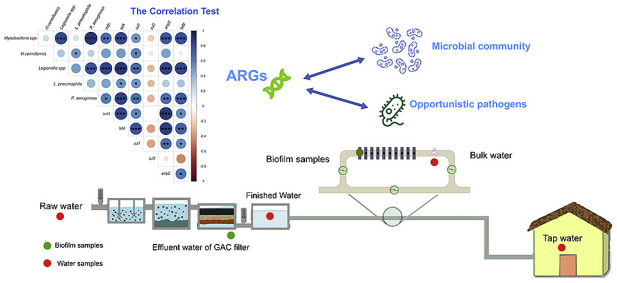
HIGHLIGHTS

- Diverse ARGs and OPs presents in drinking water and biofilms.
- Relative abundances of ARGs and OPs in biofilms were higher.
- Relative concentrations of OPs correlated well with the ARGs and *int*I1.

GRAPHICAL ABSTRACT



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ABSTRACT

The emergence of antibiotic resistance genes (ARGs) and opportunistic pathogens (OPs) in drinking water system posed potential risks to human health. However, the occurrence of ARGs and OPs in drinking water biofilms is still at its infancy. In this study, we investigated the occurrence of ARGs and OPs in both water and biofilm samples from a drinking water system, and the correlation between ARGs and microbial communities was analyzed. The quantitative PCR results showed that the drinking water treatment process effectively decreased the absolute abundances of ARGs. However, the relative concentration of ARGs did not show a significant difference between raw water and treated water samples. Compared with bulk water and tap water samples, biofilms had higher relative abundances of ARGs. 16 S Illumina Miseq sequencing results showed that microbial communities of biofilms were distinguished with water samples. Meanwhile, qPCR results of OPs also showed that biofilms had higher relative abundances of OPs compared with water samples. Furthermore, the Spearman correlation analysis indicated that *Dechloromonas*, *Desulfovibrio*, *Methylobacterium* and *Propionivibrio* correlated well with the relative abundance of ARGs. The absolute concentrations of OPs and ARGs also showed a significant correlation. Results of this study suggest that biofilms could serve as the reservoirs for the spread of ARGs and the interaction between biofilms and bulk water requires further research.

1. Introduction

The occurrence and spread of antibiotic resistance genes (ARGs)

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are pressing public health problems worldwide (Oberle et al., 2012). ARGs encoding antibiotic resistance are considered as emerging contaminants in aquatic environments (Gao et al., 2012; Pruden et al., 2006). Considering its impact on human-associated microbiota, the occurrence of ARGs and its spreading into drinking water should arise extensive attention. Recently, findings on ARGs in drinking water systems, especially in treated water that have direct contact with human-beings have given rise to concerns from both researchers and the public (Garner et al., 2018; Hao et al., 2018; Ma et al., 2017b; Su et al., 2018; Xi et al., 2009). It has been proved that the drinking water treatment process could not remove all the microorganisms, leading to the regrowth of disinfection resistant bacteria in drinking water distribution systems (DWDSs) (Liu et al., 2014). Several studies have reported that chlorine disinfection can select antibiotic resistant bacteria and increase the relative abundance of ARGs (Jia et al., 2015; Liu et al., 2018). Therefore, the occurrence of ARGs in DWDS and plumbing system may pose potential risks and deserve more public attention.

Biofilms have become a hotspot for the spread of antibiotic resistance genes in drinking water systems. In DWDSs, more than 90% of the total biomass can be found in biofilms growing attached to pipe walls (Flemming H C, 2002). In these biofilms, bacteria live in an environment with high cell density and close distance between bacteria, compared to planktonic bacteria (Flemming et al., 2016), which could facilitate the transmission of antibiotic resistance since horizontal gene transfer (HGT) tends to happen with dense bacteria populations (Krol et al., 2013; Zheng et al., 2018). Moreover, extracellular polymeric substances (EPS), in which biofilm cells are embedded, protect microorganisms from various adverse agents like disinfectants and shearing force (Flemming and Wingender, 2010). Previous studies have demonstrated that EPS produced by microorganism intensified the aggregation of bacteria under high chlorine condition in DWDS, hence increased the chlorine-resistance capability (Xing et al., 2018; Xue et al., 2012). Owing to the convenience for bacteria growth, biofilms have been considered as the environmental reservoir of opportunistic pathogens and represented a potential source of drinking water contamination (Waak et al., 2018; Wingender and Flemming, 2011). Moreover, non-pathogenic antibiotic resistant bacteria (ARB) in biofilms could spread ARGs to opportunistic pathogens through HGT, posing threats to human health. However, the relationship between OPs and ARGs in biofilm has not been established.

Researches about antibiotic resistance in drinking water often focused on individual components, such as source water (Bai et al., 2015; Jiang et al., 2013), drinking water treatment process and DWDS (Garner et al., 2018; Xu et al., 2016), instead of the full-scale drinking water system. Although the occurrence of ARGs in different parts of the drinking water system was confirmed, how ARGs change along the full-scale drinking water system is still unknown. Former studies investigated the removal effect of the traditional and advanced treatment process on the concentration of ARGs as well as their occurrence in tap water (Xu et al., 2016), without the existence in biofilms and bulk water of DWDS. It has been proved that the detachment of biofilms would impact the microbial community and ARGs in tap water (Zhang et al., 2018a, 2018b). Therefore, understanding how biofilms impact the spread of ARGs and the occurrence in bulk water is extremely important.

The aim of the research reported here was to investigate the existence of ARGs and OPs in drinking water and biofilms. A special biofilm sampling device was applied to attain both biofilm and water samples in DWDS. The potential link between the biofilm bacterial community and associated ARGs in biofilm and water samples was studied. The results of this study can provide a better understanding of the prevalence and transmission of ARGs in drinking water systems.

2. Methods and materials

2.1. Drinking water treatment plant and distribution system

Biofilm and water samples were collected from a representative drinking water treatment plant (DWTP) and the corresponding drinking water distribution system in October 2018 in an eastern city of China. In this plant, raw water from the Yangtze River was treated by clarification and filtration, followed by ozonation and granular active carbon (GAC) filtration as well as UV disinfection. The detailed information of the treatment process was presented in supplementary material. Then, monochloramine was added with a final concentration at 1.1 mg/L to prevent bacteria regrowth in DWDS. The pipes of the DWDS are made of cast iron, ductile cast iron as well as steel, and construction dates range from 1980 to 2009, with inner diameters of over 100 mm. The relative positions of DWTP and sampling points were illustrated in Fig. 1a.

2.2. Sampling

Water samples from the water source (RW), after GAC filtration (GW), and after disinfection (FW) were collected. About 5 L water sample was collected in a sterilized glass bottle. Two biofilm samples from the GAC filter (GB1, GB2) were collected at a depth of 50 cm. Approximately 30 g GAC was added to 100 mL sterile phosphate buffered solution (PBS) and was shaken ultrasonically (40 kHz) for 20 min to suspend the bacteria of biofilm (Zheng et al., 2018). Three sampling points (A, B and C) were selected in this DWDS (Fig. 1a). Biofilm and bulk water samples were available with special biofilm sampling devices (Fig. 1b). Briefly, the device consisted of a 150 cm diameter cast iron pipe to cultivate biofilms as well as a by-pass pipe. Ten biofilm coupons were installed on the side of the sampling pipe and retained the curvature of the pipe to prevent disturbing the flow characteristics. The bulk water (DW1, DW2, DW3) was sampled at the taps equipped with the pipe before biofilm sampling using. Dust iron coupons were placed in the device in December 2014 and remained in the actual DWDS for 5 years to obtain mature biofilms. While sampling biofilms, the valve on the sampling pipe was closed with the by-pass pipe opened. Then biofilm coupons were removed from the pipe and placed carefully in 250 mL sterilized stainless-steel containers filled with sterilized PBS. Two biofilm coupons were collected in site A (DB1, DB2) and site B (DB3, DB4), while only one biofilm coupon was obtained in site C (DB5). Tap water samples (TW1, TW2, TW3) were collected from apartment buildings near the above sampling points. Bulk water and tap water samples were collected in 5-L sterilized glass bottles. All these samples were transported on ice, stored at 4 °C until analysis, and processed within 6 h after sampling.

2.3. Water quality analyses

For water chemistry analyses, residual chlorine (PC II, HACH, USA) and temperature were determined on the spot. Upon arrival at the lab, pH, turbidity (2100Q, HACH, USA) and DOC (TOC-L CPH, SHIMADZU Corporation, Japan) were measured. UV spectrophotometer (UV 1800, SHIMADZU Corporation, Japan) was utilized for measuring UV_{254} with a quartz cuvette (1 cm in length) at the wavelength of 254 nm. Heterotrophic bacterial counts (HPCs) were determined by samples plated on R2A agar. The number of the total colonies in the agars was regarded as HPC after incubation at 22 °C for 7 days. To overcome the weakness of culture-dependent methods which ignore unculturable bacteria, total cell concentration (TCC), and intact cell concentration (ICC) were performed by flow cytometry, according to van der kooij's study (van der Kooij

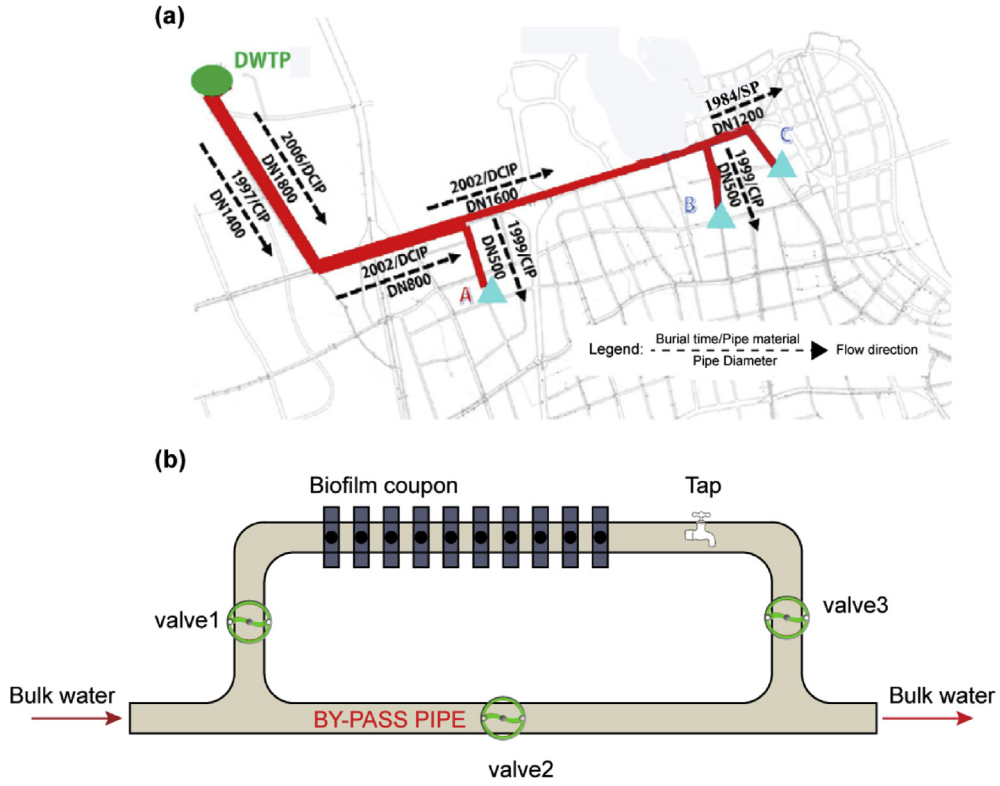

Fig. 1. The relative location of sampling points in the drinking water system (a) and the structure of the biofilm sampling device (b). In usual, valve1 and valve3 were open with valve2 closed. While sampling biofilm, valve1 and valve3 were closed but valve2 was open to let water go through the by-pass pipe. Abbreviations: CIP (Cast-Iron Pipe), SP (Steel Pipe), DCIP (Ductile Cast-Iron Pipe).

and Veenendaal, 2014). Briefly, 500 μL of a water sample was blended with 5 μL SYBR green (100 × dilution of a 10,000 × concentrate) (Life Technologies Ltd., USA) and 5 μL propidium iodide (50 g/mL) (Life Technologies Ltd., USA) and stained for 15 min at 25 °C. Subsequently, the membrane-intact and membrane-disrupted cells were counted by FAC SCalibur flow cytometer (BD Biosciences, USA). The results were expressed in cells per milliliter (cells/mL).

2.4. Sample pretreatment and DNA extraction

To detach biofilms, coupons were shaken with an amplitude of 13 for 2 min by an ultrasonic processor (S-4000, SONICATOR Company, USA). Approximately 50 mL of PBS containing turbid biofilms was obtained after the biofilm was pretreated. Then both biofilm and water samples were filtered through 0.22 μm pore-size cellulose ester filters (Millipore, Billerica, MA). DNA was extracted using the DNeasy® PowerWater® Kit (QIAGEN, USA) according to manufacturer protocol and was then stored at −80 °C until further processing. DNA quantification and purification were determined by spectrophotometry using NANODROP (DS-11, DeNovix, USA).

2.5. Quantitative PCR analysis

ARGs encoding the resistance to sulfonamides (*sul*1, *sul*2), tetracyclines (*tet*A, *tet*M), β-lactams (*amp*C) as well as the integrase gene of class I integron (*int*I1) were quantified using SYBR Green I qPCR in ABI sequence detection system 7500 (Applied Biosystems, USA). The primer sequences and reaction conditions are compiled in Supplementary Table S1. The qPCR mixtures and protocol conditions were detailed in the supplementary information. All qPCR analyses were performed in triplicate with DNA-free water as negative controls.

To determine the existence of opportunistic pathogens in drinking water and biofilm samples, *Mycobacteria* spp., *Legionella* spp., *L. pneumophila*, *Pseudomonas. aeruginosa*, *Hartmannella vermiformis* and bacterial 16 S rRNA genes were determined by qPCR assays using previously reported methods (Li et al., 2018). The primer sequences and reaction conditions were shown in Table S1. The limit of quantification (LOQ) for all qPCR assays was 10 gene copies/reaction, except for 16s rRNA genes (100 gene copies/reaction). Information about qPCR mixtures and protocol conditions was presented in the supplementary information.

2.6. Amplicon targeted sequencing of 16 S rRNA genes and analysis

Bacterial 16 S rRNA genes were amplified with barcoded primer 338 F/806 R (338 F: ACTCCTACGGGAGGCAGCAG, 806 R: GGACTACHVGGGTWTCTAAT). PCR reactions were performed in triplicate as previously reported (Zhang et al., 2018b). After amplification, PCR products were detected on 2% (w/v) agarose gels, and the minimum size of each amplicon was 550 bp. PCR amplicons were extracted and purified by the AxyPrep DNA gel extraction kit (Axygen Biosciences, Union City, CA, USA), then quantified by QuantiFluor™-ST (Promega, USA). The purified amplicons were pooled in equimolar amounts, and then were paired-end sequenced (2 × 250) by Majorbio Co., Ltd. in Shanghai using the MiSeq PE300 platform (Illumina, USA). Raw sequences were demultiplexed and quality filtered by USEARCH 7.1 (http://drive5.com/uparse/), in which the sequence containing > 3 consecutive bases or obtaining a quality score < 20 were removed. And sequence with chimera were also ruled out. After filtration of low-quality sequence, the optimized sequences were clustered into operational taxonomic units (OTUs) using UPARSE 7.1, with a limit

of 3% distance (equivalent to 97% sequence similarity). Subsequently, effective sequences were aligned against SILVA database (http://www.arb-silva.de), and then were identified down to different levels using Ribosomal Database Project (RDP, http://rdp.cme.msu.edu/) Bayesian classifier at 70% threshold.

2.7. Statistical analysis

Non-metric multidimensional scaling (NMDS) was performed to evaluate the difference of microbial community among the samples based on Bray-Curtis distance created by Qiime 1.7.0 (http://qiime.org/index.html). Wilcoxon rank-sum test was carried to assess the difference between water and biofilm samples at 95% confidence intervals. Bivariate correlation (2-tailed) analysis using Spearman methods was performed to explain the correlation between ARGs and microbial community composition. All statistical analyses were performed on SPSS 23.0. The p-value <0.05 was statistically significant, and $p < 0.01$ meant that the difference was more significant in statistics.

3. Results

3.1. ARGs in the biofilm and water samples

The presence of five common ARGs and class I integron (*intI1*) was determined for both water and biofilm samples in the full-scale drinking water system (Table.S3). In water samples, ARGs with high absolute concentration included *tet*A (ranging from 2.42×10^4 to 1.38×10^5 copies/mL) and *sul*1 (ranging from 1.57×10^3 to 2.01×10^5 copies/mL), which were detected in all samples. After the drinking water treatment process, absolute concentrations of all ARGs were reduced, with removal rates ranging from 66.85% to 100%. However, it was observed that the absolute concentration of *tet*A, *sul*1 and *tet*M increased again in DWDS and remained at high levels in tap water. The concentration of *intI*1 ranged from 1.31×10^4 to 2.74×10^5 copies/mL in all water samples. In biofilm samples, the absolute concentration of gene *intI*1 ranged from 8.65×10^6 to 4.87×10^7 copies/cm^2. *Tet*A, *sul*1 and *amp*C were also detected in all biofilm samples, while *sul*2 and *tet*M had a detection frequency of 40% and 80%, respectively. The correlation analysis between ARGs and water quality parameters indicated that the concentration of *tet*M was significantly correlated with the DOC, ICC and TCC (Spearman, $p < 0.05$, $n = 9$), while the correlations between other ARGs and water quality parameters were not observed.

Relative concentrations of ARGs normalized by 16 S rRNA genes are shown in Fig. 2. Biofilm samples had higher relative concentrations of *intI*1 compared to water samples ($p < 0.01$). *Tet*A, *sul*1, *sul*2, *amp*C and *tet*M did not show significant differences between water and biofilm samples ($p > 0.05$). *Tet*M was the most dominant ARG in both water and biofilm samples, of which the average relative concentrations were 5.19×10^{-3} copies/16 S rRNA genes and 1.18×10^{-2} copies/16 S rRNA genes, respectively. Although the drinking water treatment process reduced the absolute concentration of ARGs, it was observed that the relative concentration of ARGs did not show significant differences between raw water and finished water samples ($p > 0.05$).

3.2. Bacterial community in biofilm and water samples

The 16 S rRNA sequencing approach revealed diverse bacterial communities in both water and biofilm samples. The raw sequence reads ranged from 43,757 to 76,870, and the least number was adopted to subsample sequences for comparing different samples at the same sequencing level. According to the type (water or biofilm) and origin (DWTP, DWDS, or tap), samples were divided to five groups, namely water samples in the DWTP, biofilm samples of GAC, bulk water samples, biofilm samples in DWDS and tap water, of which the average OUT numbers were 747, 976, 898, 1404 and 1610, respectively (Fig.S1). Water samples had higher OTU numbers compared with biofilm samples, while biofilm samples had more unique OTUs (14.9% of the total OTU were unique among all samples). Tap water samples had the lowest OTU numbers compared with water samples in the DWTP and DWDS. The alpha diversity indices of water and biofilm samples did not show a significant difference (Fig. S2), such as the Shannon index ($p > 0.05$), Simpson index ($p > 0.05$) and Chao index ($p > 0.05$). Previous studies also reported lower diversity and evenness in the biofilm community compared with suspended bacteria (Stuken et al., 2018; Waak et al., 2019; Ling et al., 2016; Henne et al., 2012), which could be attributed to the discrepancy of the growth environment and mobility between biofilm and bulk water. Bacteria in bulk water have higher mobility and come from various origins, including the treatment plant, water source and detached biofilms. However, bacteria predominant in biofilms can actively contribute to the succession of biofilms, while bacteria that cannot adapt to the biofilm mode vanished over time (Henne et al., 2012).

The microbial community composition at phyla level was presented in Fig.S3. The *Proteobacteria* was the most abundant phylum in all samples except raw water, with a wide range of 36.60%–94.52%. *Actinobacteria* (39.02%) and *Cyanobacteria* (31.97%) were only dominant in raw water samples. After the drinking water treatment process, the proportion of *Actinobacteria* decreased to 1.36% in finished water. However, both biofilm samples and bulk water samples in DWDS had high proportions of *Actinobacteria* (8.05%–38.24%), which indicated *Actinobacteria* could adapt to the circumstance and regrow in drinking water pipes. The proportion of *Cyanobacteria* also had a significant decrease ($p < 0.05$) after water treatment and remained at a low level in DWDS and tap water because of the dark habitat. Further comparison of microbial communities was conducted at class level (Fig. 3a). Within phylum *Proteobacteria*, *α-proteobacteria* represented 22.34%–66.79% in water group, followed by β-*Proteobacteria*, γ- *Proteobacteria* and δ-*Proteobacteria*. In comparison, the most abundant taxa of biofilm samples were δ-*Proteobacteria* with a proportion of 18.86%~46.12%. β-*Proteobacteria* in biofilm samples and tap water samples had higher proportions of 5.43%–44.79 and 11.76%–53.02%, respectively, while bulk water samples only had a proportion of 1.64%–9.09% of β-*Proteobacteria*. *Cyanobacteria* and *Flavobacteria* also showed differences between biofilm and water samples ($p < 0.05$). Microbial community profiles at genus level were presented by a heatmap (Fig. 3b). Both top 20 classified genera and 16 samples were hierarchically clustered based on Bray-Curtis similarity index. It can be observed that biofilm samples and water samples were distinguished. The most representative bacterial genera in water samples were *Methylobacterium*, *Nitrosomonas*, *Phreatobacter* and *Sphingomonas*. In biofilm samples, *Desulfovibrio*, *Mycobacterium*, *Nitrosomonas* and *propionivibrio* were dominant genera. Tap water samples, biofilm and bulk water samples shared approximately 70% of genera. However, biofilm samples had a higher proportion of *Desulfovibrio* (18.36%–45.97%), which was not detected in other samples.

3.3. Opportunistic pathogens in biofilm and water samples

Representative OPs in drinking water including *Mycobacteria* spp., *Legionella* spp., *L. pneumophila*, *P. aeruginosa* were determined by qPCR (Table.S4). *Mycobacteria* spp., *Legionella* spp., *L. pneumophila* and *P. aeruginosa* were quantified in all samples with concentrations ranging from 9.62×10^3 to 2.49×10^6 copies/mL in

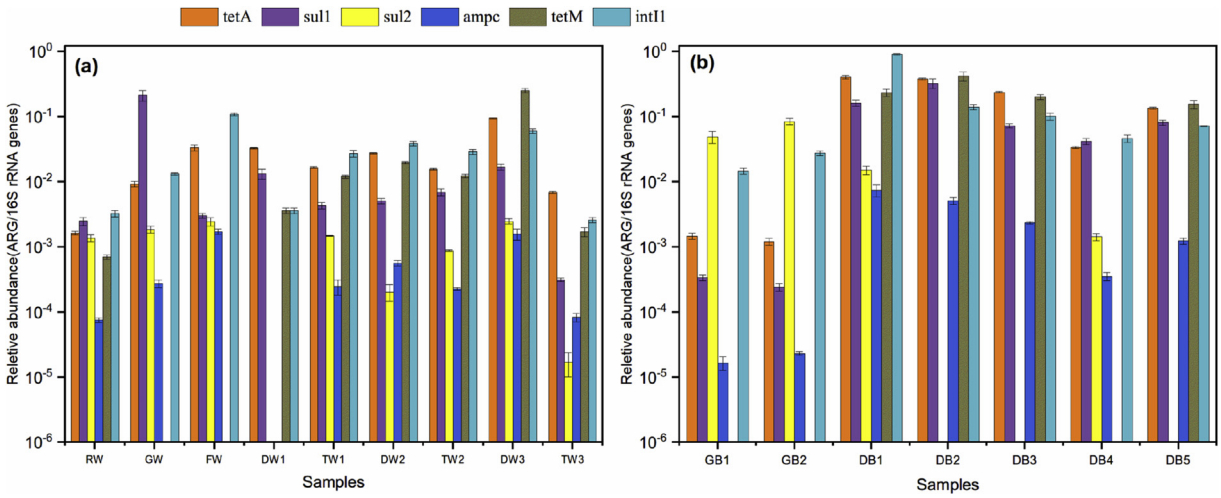

Fig. 2. The relative abundances of ARGs and *intI*1 in water and biofilm samples. RW: raw water; GW: water after GAC filtration; FW: finished water of the DWTP; DW: bulk water in DWDS; TW: tap water; GB: GAC biofilms; DB: biofilms in DWDS. The error bar indicated the standard deviation.

water samples and 9.87×10^6 to 2.72×10^7 copies/cm^2 in DWDS biofilm samples. The *Mycobacterium* genus was tracked as representative of Nontuberculous mycobacteria (NTM) species (Wang et al., 2015). The absolute concentration of *Mycobacterium* was reduced during water treatment while the relative abundance remained at high levels (Fig. 4). *Mycobacterium* in biofilm samples were more abundant with the average relative abundance of 2.76×10^{-2} to 4.09×10^{-1} copies/16 S rRNA genes. *L. pneumophila* was only detected in raw water, bulk water samples in Site A and tap water in site B among all water samples, with concentration from 7 to 17 copies/mL. In comparison, *L. pneumophila* was quantified in all biofilm samples, with concentration from 1.11×10^3 to 2.13×10^4 copies/cm^2. In addition, the average relative concentration of *P. aeruginosa* in biofilms was 2.23 folds higher than that in bulk water and tap water samples. *H. vermiformis* is not itself a pathogen, but is viewed as an important host for opportunistic pathogen amplification in drinking water system (Li et al., 2018). *H. vermiformis* had a detection frequency of 44.4% in water samples and were nondetectable in tap water samples. In comparison, *H. vermiformis* was positive in all biofilms samples and the relative concentration ranged from 1.89×10^{-7} to 4.04×10^{-5} copies/16 S rRNA genes.

4. Discussion

4.1. The prevalence of ARGs in the drinking water system from source to tap

In the present study, we measured seven ARGs in a full-scale water treatment system, and five of these molecules were detected in all the collected samples. It was found that all detected ARGs showed a decrease of absolute concentration in the finished water (Table.S3). The disinfection process in DWTP can eliminate bacteria as the total 16 S rRNA genes in finished water was significantly lower than that in raw water (Table S2). As the host for ARGs, the reduction of microorganisms could result in decreases in absolute concentration of ARGs. However, it was reported that water distribution system had the greatest potential for the enrichment of ARGs (Su et al., 2018; Xi et al., 2009). Since microorganisms can regrow in DWDS with suitable conditions (van der Kooij and Veenendaal, 2014), the bacteria carrying ARGs can proliferate in water and biofilm phase, leading to increases in the absolute concentration of ARGs. Previous studies combining high-throughout sequencing and metagenomic approaches have demonstrated that chlorination can alter antibiotic resistome in drinking water via bacterial community shift (Jia et al., 2015, 2020). In this drinking water system, the average absolute concentration of all ARGs in tap water samples except *ampC* increased compared to the finished water, which indicated DWDS could play an important role in the spread of ARGs in drinking water.

As to DWDS, recent studies have indicated that biofilms could contribute to the spread of ARGs, mostly as a result of the high cell density and close cell-to-cell proximity (Liu et al., 2016). The tested ARGs including *tet*A, *sul*1, *sul*2, *amp*C and *tet*M showed higher relative abundance in biofilm samples compared to water samples ($P < 0.05$). This is in accordance with a recent study which also reported that *erm*A, *erm*B, *aph*A2, *sul*2 and *tet*O showed higher relative abundance in biofilm phase after a 120-d cultivation (Zhang et al., 2018c). Moreover, relative abundances of *intI*1 in biofilm samples were significantly higher than those in water samples ($p < 0.05$). The biofilm mode of life in DWDS has ecological advantages over suspending mode, including biodiversity, gene pool and facilitated genetic exchange, protection against biocides and other types of stress in a high-density population (Flemming, 2009). It has been confirmed that rates of HGT are typically higher in biofilms than in planktonic cultures (Madsen et al., 2012), and increased transfer of antibiotic resistance determinants on mobile genetic elements was noted in biofilm of various bacteria, such as *Staphylococcus aureus* (Savage et al., 2013).

4.2. Comparing the bacteria community between water and biofilm samples

Research efforts have revealed that the identity and composition of microbial communities in drinking water biofilms are different in comparison to the corresponding planktonic population in bulk water (Henne et al., 2012; Liu et al., 2014). In the present study, the NMDS was conducted to compare the microbial community in raw water, water in DWTP, bulk water and biofilms in DWDS as well as tap water (Fig. 5). As shown, microbial communities of biofilms were significantly distinguished from other samples. *Desulfovibrio*, which is known as sulfate-reducing bacteria, is commonly associated with microbiologically influenced corrosion and corroded, iron-rich environments (Gomez-Smith et al., 2015; Ren et al., 2015). In this study, *Desulfovibrio* occupied a high proportion in microbial community of biofilm samples but was not detected in bulk water

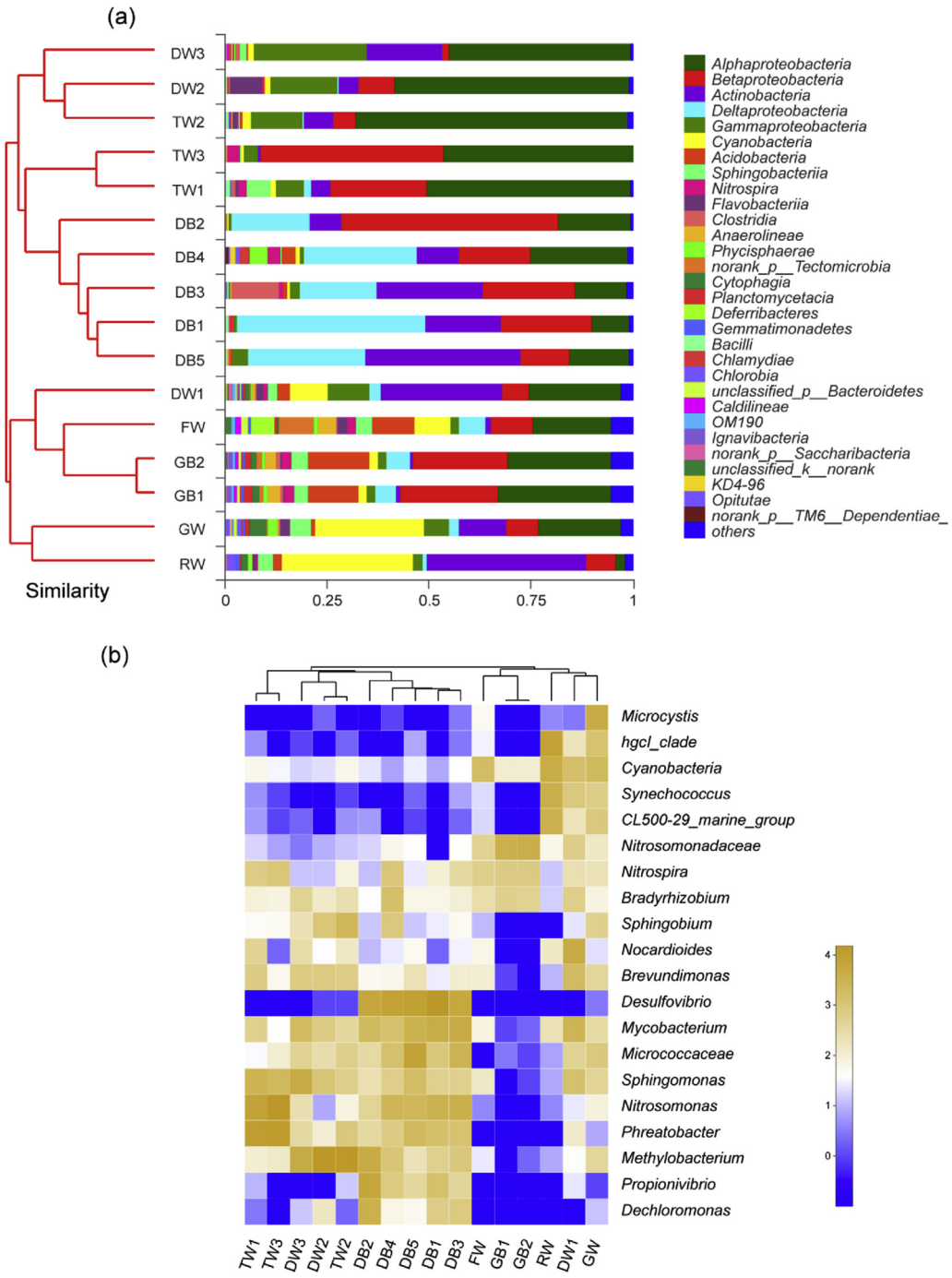

Fig. 3. Microbial community composition at class level (a) and the heatmap of microbial community composition at genus level (top 20). The others refer to classes less than 0.01.

samples. The higher relative abundance of *Desulfovibrio* in biofilms may be attributed to sulfate in the treated water from coagulation with aluminum sulfate ($Al_2(SO_4)_3$) and the iron corrosion in the coupons which could provide the electron donor for these organisms from the reduction of protons. The prevalence of *Desulfovibrio* in DWDS and their relation to the corrosion suggests that attempts to limit their growth, such as reducing the supply of sulfate, may be beneficial for the maintenance of DWDSs. In addition, the proportion of *Mycobacteria* in biofilms was significantly higher than that in bulk water. Previous studies reported that Mycobacteria-like OTUs were prominent in biofilms growing on the surface of water mains within a chloraminated DWDS (Gomez-Smith et al., 2015). The q-PCR results also showed that mycobacteria had a higher relative abundance from 2.76×10^{-2} to 4.09×10^{-1} copies/16 S rRNA genes. *Mycobacteria* have a variety of advantages to proliferate in drinking water, such as the resistance to disinfectants (Zhang et al., 2018b), the ability to form biofilms and their ability to survive in oligotrophic conditions (Hall-Stoodley et al., 1999). Since environment mycobacteria include opportunistic pathogens, such as *Mycobacterium avium*, it is important to understand their occurrence in biofilms and bulk water. However, the detachment of biofilms was not clear and more research is needed to determine how biofilms impact the microbiome and other characteristics of bulk water.

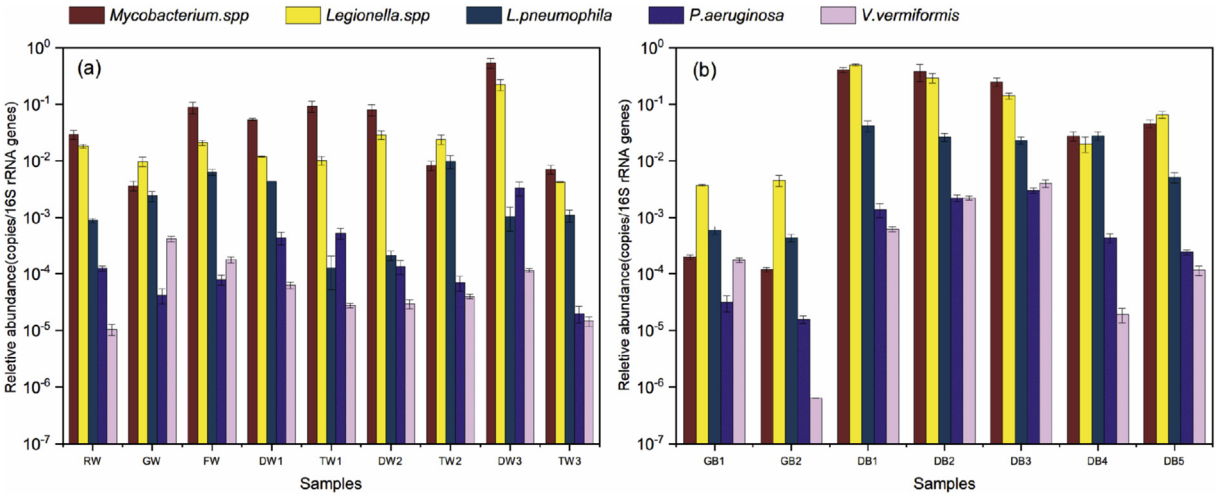

Fig. 4. Relative concentration of OPs in water and biofilm samples. The error bar indicated the standard deviation.

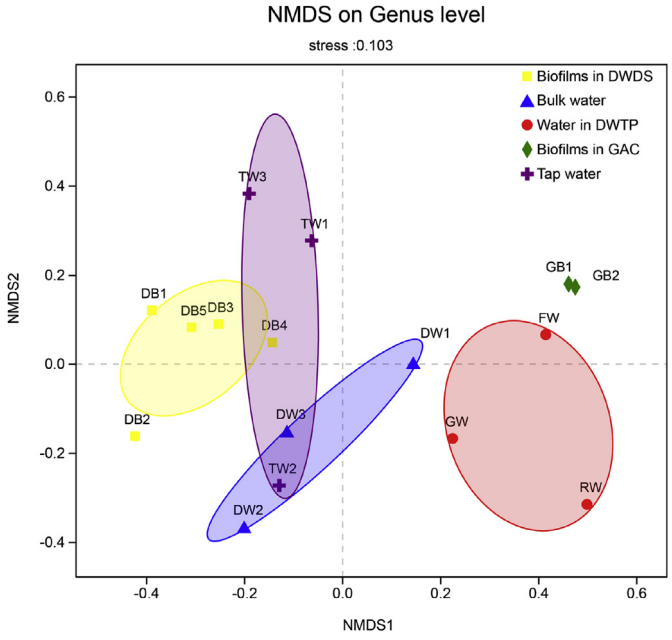

Fig. 5. NMDS representations of the microbial community in the full-scale drinking water system. NMDS ordination was derived from pairwise Bray-Curtis distance.

4.3. The effect of microbial communities on ARGs in biofilm and water samples

Previous studies have revealed that bacterial community shift plays an important role in shaping the antibiotic resistome in drinking water (Jia et al., 2015; Zhang et al., 2018a). Different processes including water treatment and distribution could impact the microbial community composition, and, therefore, change the abundance of antibiotic resistome. Bacteria in biofilms exhibit different life styles compared to the free-living bacterial cells and consequently comprise different core populations (Ling et al., 2016). The correlation analysis between the abundance of bacteria genus and the relative abundance of ARGs revealed that *Dechloromonas*, *Desulfovibrio*, *Methylobacterium*, and *Propionivibrio* were correlated with ARGs detected in this study (Fig.S4). *Dechloromonas*, which was related to the denitrifying process, are often found in soils and freshwater sediments (Han et al., 2019). *Dechloromonas* were found to significantly correlate with the relative abundance of ARGs except *sul*2 in this study ($p < 0.01$, $n = 16$). *Desulfovibrio*, which occupied high proportions in bacterial community of biofilm samples, were also found to correlate with the relative abundance of *tet*A, *sul*1, and *amp*C. Moreover, *Mycobacterium* showed significant correlations with all ARGs, especially *tet*A, *sul*1 and *tet*M ($p < 0.001$, $n = 16$), which indicated that bacteria in the genus of *Mycobacterium* might be the host of these ARGs. Previous research using the metagenomic assembly-based host-tracking analysis has identified that *Mycobacterium* can be the host of ARGs in tap water (Ma et al., 2017b). Since the *Mycobacterium* encompasses opportunistic pathogens, the high frequency of ARGs carried by *Mycobacterium* in drinking water may increase the risk of infection and antibiotic ineffectiveness in human beings.

Since the 16 S rRNA sequencing cannot provide the information of bacteria species, the qPCR targeting the representative OPs was used to reveal the relationship between OPs and ARGs. The correlation analysis showed that the relative concentrations of OPs were significantly correlated with the concentration of the ARGs and *int*I1 (Fig. 6). *Mycobacteria* spp. and *Legionella* spp. were positively correlated with the relative concentration of *tet*A, *sul*1, *amp*C and *tet*M, which was in accordance with the results of 16 S rRNA sequencing ($r = 0.50–0.88$, $p < 0.05$). *L. pneumophila* was correlated to the relative concentration of *tet*A, and *sul*1 ($p < 0.05$). Moreover, *int*I1 correlated well with *Mycobacteria* spp., *Legionella* spp. and *P. aeruginosa*. Integrons could contribute to the spread of antibiotic resistance by facilitating lateral ARG transfer and incorporation into bacterial chromosomes (Ma et al., 2017a). The high correlation between OPs and *int*I1 might pose risks in biological safety in drinking water, and the pathway how OPs obtain ARGs and mobile genetic elements requires further research.

5. Conclusions

This study reports the occurrence of ARGs and OPs and the correlation between ARGs and microbial community in drinking water and biofilms. A variety of ARGs and OPs were detected in both water and biofilm samples, indicating the prevalence of ARGs and OPs in drinking water system. The results demonstrated that biofilms contained higher abundances of ARGs and OPs than those in bulk water, and hence had higher risks for the dissemination of ARGs in drinking water system. The correlation between microbial

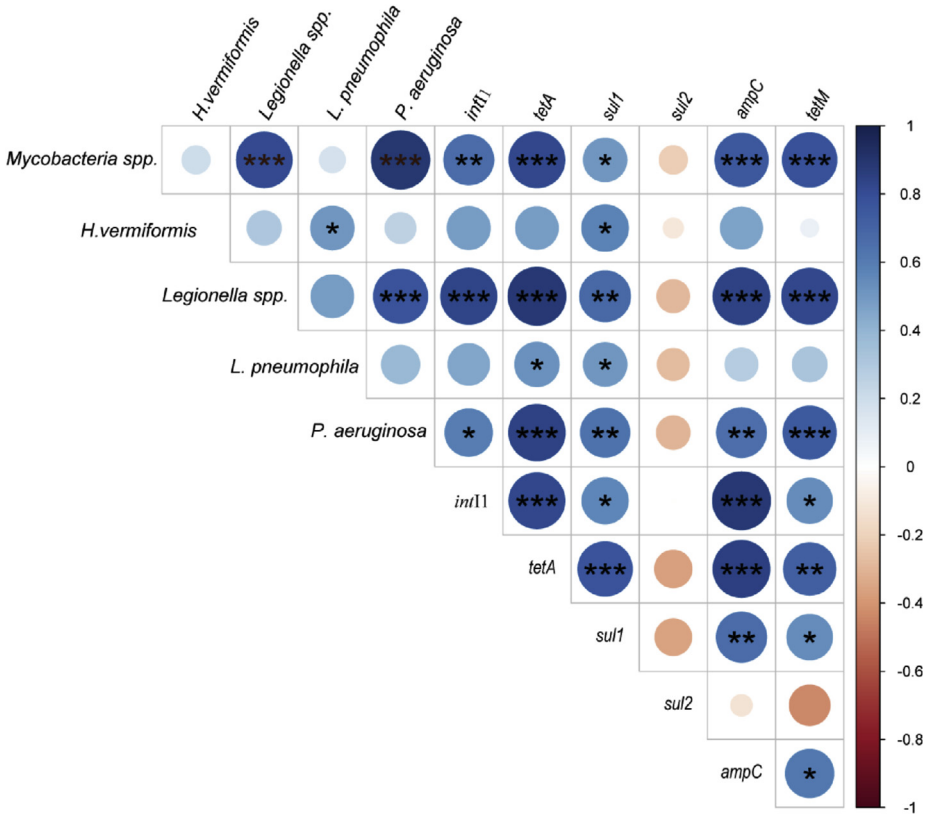

Fig. 6. Heatmap of pair-wise Spearman's correlation coefficients computed between the relative concentration of targeted genes (*: P < 0.05, **: P < 0.01, ***: P < 0.001).

community and the abundance of ARGs revealed that the change of the bacterial community may be a major driver in shaping ARG profiles, propagation, and distribution in drinking water. Moreover, the relative concentrations of OPs were significantly correlated with the concentration of the ARGs and intI1. The results of this study provided the evidence for the prevalence of ARGs and OPs in drinking water and biofilms and further studies are needed to identify the interaction between biofilms and bulk water and the influence on the dissemination of ARGs and ARB in drinking water system.

Credit author statement

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Declaration of competing interest

The authors declare no competing financial interests related to the publication of this study.

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Appendix A. Supplementary data

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