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High-throughput profiling of antibiotic resistance genes in drinking water treatment plants and distribution systems☆

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ABSTRACT

Antibiotic resistance genes (ARGs) are present in surface water and often cannot be completely eliminated by drinking water treatment plants (DWTPs). Improper elimination of the ARG-harboring microorganisms contaminates the water supply and would lead to animal and human disease. Therefore, it is of utmost importance to determine the most effective ways by which DWTPs can eliminate ARGs. Here, we tested water samples from two DWTPs and distribution systems and detected the presence of 285 ARGs, 8 transposases, and *intI*-1 by utilizing high-throughput qPCR. The prevalence of ARGs differed in the two DWTPs, one of which employed conventional water treatments while the other had advanced treatment processes. The relative abundance of ARGs increased significantly after the treatment with biological activated carbon (BAC), raising the number of detected ARGs from 76 to 150. Furthermore, the final chlorination step enhanced the relative abundance of ARGs in the finished water generated from both DWTPs. The total enrichment of ARGs varied from 6.4-to 109.2-fold in tap water compared to finished water, among which beta-lactam resistance genes displayed the highest enrichment. Six transposase genes were detected in tap water samples, with the transposase gene TnpA-04 showing the greatest enrichment (up to 124.9-fold). We observed significant positive correlations between ARGs and mobile genetic elements (MGEs) during the distribution systems, indicating that transposases and *intI*-1 may contribute to antibiotic resistance in drinking water. To our knowledge, this is the first study to investigate the diversity and abundance of ARGs in drinking water treatment systems utilizing high-throughput qPCR techniques in China.

1. Introduction

Antibiotic resistance genes (ARGs) are emerging environmental pollutants (Pruden et al., 2006). ARGs are present in wastewater treatment plants (WWTPs), livestock and soil. They can enter into surface water and underground water through rain or surface runoff, resulting in high levels of ARGs that are found in the water environment (Wellington et al., 2013). Additionally, the association of ARGs and mobile gene elements (MGEs) can accelerate the proliferation of ARGs through horizontal gene transfer (HGT) in the water environment. HGT is widely recognized as the mechanism responsible for the widespread distribution of bacterial antibiotic resistance (de la Cruz and Davies, 2000; Gyles and Boerlin, 2014).

WWTPs are an important source of introducing ARGs into surface water. ARGs remaining in the finished water from WWTPs have a good chance of entering into rivers or lakes, contributing to antibiotic resistance pollution in surface water (Amos et al., 2014; Xu et al., 2015; Zhang et al., 2009). Also, aquaculture is the most direct way of introducing ARGs into the water environment. Antibiotic resistance levels in aquaculture systems are well documented and it has been suggested that this system may serve as a reservoir for antibiotic-resistant bacteria (ARB) and ARGs (Gao et al., 2012; Phuong Hoa et al., 2008; Su et al., 2011). In modern cities, the drinking water that is supplied to the population is often obtained from nearby surface water after its rigorous treatment in WWTPs. However, the high concentration of antibiotics and ARGs remaining in surface water might enter water supply pipelines through drinking water treatment systems(Jones et al., 2005), and this increases the potential for antibiotic resistance pollution of drinking water. The finished water from drinking water treatment plants (DWTPs) is provided to the local population through water supply

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systems. Thus, the quality of the drinking water can potentially cause disease to the citizens who consume the water.

Jiang et al. investigated the ARGs present in the Huangpu River and found that the abundance of ARGs in water samples near the drinking water sources was higher than other samples that were distant from those sources (Jiang et al., 2013). DWTPs have an implication on the behavior of ARGs as it might increase the antibiotic resistance of surviving bacteria, and the finished water from DWTPs have been demonstrated to contain ARGs (Guo et al., 2014; Pruden et al., 2006; Xi et al., 2009). The conventional treatment process to generate drinking water includes coagulation, sedimentation, sand filtration and chlorination. The second generation of drinking water treatment adopts the combination of ozone and biological activated carbon (BAC). Thus far, the research of ARGs in municipal works is mainly focused on WWTPs, and there is a lack of knowledge about the abundance of ARGs in urban DWTPs. Moreover, the existing research on ARGs in DWTPs is incomplete and does not reflect a comprehensive profile of ARGs in drinking water. The aforementioned influence of ARGs on human health necessitates a comprehensive investigation of ARGs in DWTPs and distribution systems.

The Qiantang River is an important commercial artery passing through the provincial capital Hangzhou before flowing into the East China Sea. To insure the safety of drinking water in this metropolitan area, various types of drinking water treatment process are employed, including conventional and advanced treatment processes. In this study, we investigated ARGs in two representative drinking water treatment plants and the distribution systems in Hangzhou City. Both drinking water suppliers use Qiantang River as the drinking water source for over 15 years and different technologies are employed during the treatment processes. In total, 285 ARGs, 8 transposes, and *intI*-1 were detected by using high-throughput quantitative PCR. This technique can be used to comprehensively profile ARGs in different environmental samples (Wang et al., 2014). The main goals of the current study are to detect the abundance of ARGs in drinking water treatment plants and distribution systems, as well as to analyze the potential for ARGs to propagate in DWTPs and water distribution systems.

2. Materials and methods

2.1. DWTPs and sample collection

Water samples from two representative drinking water treatment plants (DWTPs) were collected in June and November of 2014 in Hangzhou city, eastern China. DWTP-1 employs the advanced O_3/BAC treatment while DWTP-2 adopts conventional treatment. The water supply capacities are 100,000 m^3/d and 600,000 m^3/d in DWTP-1 and DWTP-2, respectively. The two DWTPs both use Qiantang River as the drinking water source. Qiantang River is one of the main rivers among coastal areas of southeast China. A summarization of the treatment scheme for each plant is shown in Table 1. For the analysis of drinking water distribution system, two residential areas located in the supplying area of DWTP-2 were selected in this study. The locations of DWTPs and residential areas (RA) were presented in Fig. 1.

Water samples were collected from source and finished water, as well as at every treatment procedure of the two DWTPs. The tap water from two different pipes of each residential area were also collected. Water samples were all collected using small-scale vacuum pump and suction filtration in each site due to the special properties of drinking water. Water samples were filtered through 0.22 μm membrane filters to capture bacteria and three samples were simultaneously filtered from each site. The membrane filters were carefully stored in prepared sterile silver paper bag and transported to the laboratory in an ice box.

2.2. DNA extraction

Genomic DNA from the water samples were extracted using FastDNA SPIN Kit (MP Bio, USA) according to the manufacturers' instructions. For the DNA analysis of each sample, we mixed the DNA extracted from three water samples of each site as the final DNA sample. The concentration of the purified DNA was quantified spectrophotometrically (NanoDrop ND-2000c, Thermo, USA) and stored at −20 °C until subsequent analysis.

2.3. High-throughput quantitative PCR (HT-qPCR)

All high-throughput qPCR reactions were performed using the Wafergen SmartChip Real-time PCR system. A majority of primer sets have been validated and used in previous study (Ouyang et al., 2015; Zhu et al., 2013). There were altogether 295 primer sets targeting 285 ARGs, 8 transposases, 1 class1 intergron and 16S rRNA gene. The 285 ARG assays in this research conferred resistance to almost all major antibiotics and covered three resistance mechanisms. Amplification was conducted in 100 nL reaction containing (final concentration) 1 × LightCycler 480 SYBR Green I Master Mix (Roche Inc., USA), Nuclease-free PCR-Grade water, 1 ng μL^{-1} BSA, 3 ng μL^{-1} DNA template, 1 μM of each forward and reverse primer. The thermal cycle was: initial denaturation at 95 °C for 10 min, followed by a 40 cycles of denaturation at 95 °C for 30s, annealing at 60 °C for 30s, finally with amelting curve analysis auto-generated by the program. For each primer set, amplification was conducted in triplicate and a non-template control was included.

The results of the high-throughput qPCR were analyzed using SmartChip qPCR software (V2.7.0.1), wells with multiple melting peak as well as wells with amplification efficiency beyond the range (1.8–2.2) were discarded. A threshold cycle (Ct) 31 was used as the detection limit. Only samples with three replicates that had amplification were regarded as positive. Data processing was done with Microsoft Excel 2010, while diagramming and correlation analysis were done with Origin 9.0. Heatmap graphs were produced using Heatmap Illustrator 1.0.1.

3. Result and discussion

3.1. Antibiotic resistance genes in DWTPs

3.1.1. Diversity of ARGs in DWTPs

Among all of the targeted 285 ARGs in this study, a total of 184 ARGs were detected in DWTP-1 while 192 were detected in DWTP-

Table 1
Water sources and treatment schematics of the two DWTPs targeted in this study.

DWTP	Source	Treatment processes
1	Qiantang River	Raw river[a] → Pre-ozonation[a] → Coagulation/flocculation → Sediment[a] → Sand filter[a] → Ozonation[a] → BAC[a] → Chlorination[a]
2	Qiantang River	Raw river[a] → Coagulation/flocculation[a] → Sediment[a] → Sand filter Chlorination[a]

[a] Samples were collected after each corresponding treatment process.