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Persistence of antibiotic resistance genes and bacterial community changes in drinking water treatment system: From drinking water source to tap water

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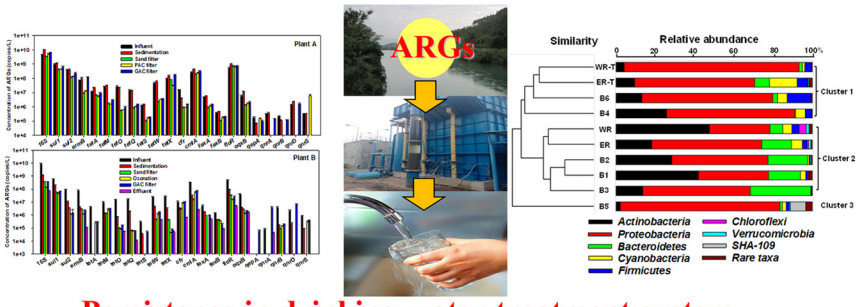
HIGHLIGHTS

- Diverse ARGs present in different types of waters.
- *Sul1*, *sul2*, *floR*, *cmlA* could be potential indicators for ARGs in the water samples.
- Sand filter and sedimentation were effective in removing ARGs.
- Granular activated carbon filtration increased the ARGs abundance.
- ARGs still existed in tap water after treatment despite significantly reduced.

GRAPHICAL ABSTRACT



Persistence in drinking water treatment system

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ABSTRACT

As emerging contaminants, antibiotic resistance genes (ARGs) have become a public concern. This study aimed to investigate the occurrence and diversity of ARGs, and variation in the composition of bacterial communities in source water, drinking water treatment plants, and tap water in the Pearl River Delta region, South China. Various ARGs were present in the different types of water. Among the 27 target ARGs, *floR* and *sul1* dominated in source water from three large rivers in the region. Pearson correlation analysis suggested that *sul1*, *sul2*, *floR*, and *cmlA* could be potential indicators for ARGs in water samples. The total abundance of the detected ARGs in tap water was much lower than that in source water. Sand filtration and sedimentation in drinking water treatment plants could effectively remove ARGs; in contrast, granular activated carbon filtration increased the abundance of ARGs. It was found that *Pseudomonas* may be involved in the proliferation and dissemination of ARGs in the studied drinking water treatment system. Bacteria and ARGs were still present in tap water after treatment, though they were significantly reduced. More research is needed to optimize the water treatment process for ARG removal.

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1. Introduction

Antibiotics are extensively used as therapeutic drugs for humans, livestock, and aquaculture, and are also feed additives in farming. In China, the estimated total antibiotic use in 2013 was 162,000 tons (Zhang et al., 2015). The extensive use of antibiotics has led to widespread environmental contamination by antibiotic residues, antibiotic resistant bacteria (ARB), and antibiotic resistance genes (ARGs), which have become a global, public concern (Pruden et al., 2006). As emerging contaminants, ARGs have been detected in a variety of environmental compartments, such as hospital wastewater (Durham et al., 2010; Vinue et al., 2010); wastewater treatment plants (LaPara et al., 2011; Zhang and Zhang, 2011); chicken (He et al., 2014), beef (Hoyle et al., 2006), pig (He et al., 2016; Xia et al., 2010), dairy (Srinivasan et al., 2005), and aquaculture farms (Ishida et al., 2010; Tamminen et al., 2011); and surface waters and sediments (Pei et al., 2006; Storteboom et al., 2010a; Storteboom et al., 2010b). ARGs can be transferred from human and animal sources to different environmental compartments, including drinking water sources and tap water, ultimately threatening human health (Becerra-Castro et al., 2015; He et al., 2016). Thus, their abundance in drinking water sources and removal in drinking water treatment plants (DWTPs) need to be understood.

Previous studies demonstrated that ARB and ARGs are prevalent in drinking water sources and distribution systems (Bergeron et al., 2015; Coleman et al., 2013; Fernando et al., 2016; Guo et al., 2014; Jiang et al., 2013; Skariyachan et al., 2015). It was reported that fecal coliform strains isolated from a major drinking water source in India were resistant to a maximum of 37 antibiotics (Skariyachan et al., 2015). Additionally, diverse ARGs providing resistance to sulfonamide, tetracycline, cephalosporin, chloramphenicol, and penicillin have been detected in drinking water sources (Bergeron et al., 2015; Fernando et al., 2016; Lyimo et al., 2016; Skariyachan et al., 2015) and DWTPs (Bai et al., 2015; Guo et al., 2014; Jiang et al., 2013). Biological activated carbon (BAC) filtration and chloramine disinfection in DWTPs could enhance the resistance of ARB against ampicillin, kanamycin, rifampicin, chloramphenicol, and streptomycin (Bai et al., 2015). Xu et al. reported that BAC filtration raised the number of detected ARGs and the final chloramine disinfection enhanced the relative abundance of ARGs in the finished water generated from the DWTPs (Xu et al., 2016). Ozone can react with organic matter and metals in water, but slightly affected bacteria and ARGs (Guo et al., 2014; Xu et al., 2016). Sand filtration increased the relative abundance of ARGs, but decreased the absolute ARGs concentrations (Xu et al., 2016). However, understanding of the removal of ARGs in DWTPs and distribution systems is still limited. Thus, understanding the variation of ARGs from the source to tap water is necessary.

The Pearl River Delta (PRD) region is a heavily populated area, and three large rivers, the East, West, and North Rivers, are the main sources of drinking water, which serves a population of >1.4 million people in Guangzhou, China. The objective of this study was to investigate the abundance of ARGs in source and tap water, the potential link between the bacterial community and ARGs, and the removal of ARGs in DWTPs in the PRD region. The ARGs selected in this investigation included tetracycline, sulfonamide, macrolide, chloramphenicol, and quinolone resistance genes, which provided resistance to the most commonly used antibiotics. The results of this study can provide better understanding of the diversity, abundance, dissemination, and removal of ARGs in the drinking water processing and distribution system.

2. Materials and methods

2.1. Study sites and sample collection

The Pearl River Delta, South China, was selected as the study area, with Guangzhou as the region's capital city. The East, North, and West Rivers are large rivers in the region, and are used as drinking water sources (Fig. S1). Due to limited accessibility for sampling, only two DWTPs, Plant A and Plant B, located in the center of Guangzhou City, were selected for investigating the occurrence and removal of ARGs in different treatment phases (Fig. S1 and Fig. 1). Detailed information of Plant A and Plant B are shown in Table S1. Plant A is a pilot plant that processes 3000 m^3/day of drinking water, sourced from the Pearl River. Plant A uses granular activated carbon (GAC) and powder activated carbon (PAC) in parallel to optimize the process parameters (Table S1 and Fig. 1). Plant B is a fully operational plant that treats up to 1,000,000 m^3/day of drinking water, sourced from the North River. Plant B consists of a sedimentation tank, sand filter, O_3 contact tank, GAC, clean water tank, and effluent (Table S1 and Fig. 1). Due to the difference in plant sizes, the two plants were not comparable. Water samples, including drinking water sources (ER, WR, and NR) and their corresponding tap water (ER-T, WR-T, and NR-T), were collected from the three rivers (East, West, and North), and from houses in Guangzhou during December 2013 and March 2014. In addition, water samples were also collected from Plants A and B; the sampling sites are presented in Fig. 1. Five and six water samples were collected from Plants A and B, respectively. A total of 17 water samples were collected in triplicate for this study.

2.2. DNA extraction and purification

For the water samples, 500 mL of each sample was filtered through a sterile membrane filter (0.45-μm pore diameter) using vacuum filtration apparatus (Luo et al., 2010; Ma et al., 2014), and the membrane filters were kept aseptically at −80 °C for total DNA extraction. DNA was extracted from the membrane filters using the PowerSoil DNA Isolation Kit (Mobio, USA), following the protocol provided by the manufacturer. DNA was further purified using the DNA Spin Kit (Tiangen, China) to minimize PCR inhibition. In total, three DNA samples were obtained for molecular analysis from each sample. The concentration and quality of the DNA samples were determined by a SmartSpec Plus Spectrophotometer (Bio-Rad, USA) and 1.5% agarose gel electrophoresis.

2.3. ARGs quantification

Quantitative PCR (qPCR) was used to quantify the abundance of 27 ARGs in water samples with an SYBR Green Real Time qPCR Kit (TaKaRa, Japan). The specific primers of the 27 ARGs and 16S rRNA genes are listed in Table S2. Positive controls contained cloned and sequenced PCR amplicons obtained from the sludge of wastewater treatment plants and manure from livestock farms, and both positive and negative controls (Milli-Q water) were included in every run. A total of 40 cycles were conducted to improve the chances of product formation from the low initial concentrations. A 20 μL PCR reaction solution was used: 10 μL of 2 × SYBR® Premix Ex Taq™ (Tli RNaseH Plus), 0.08 μL of each 0.05 mM primer, 0.04 μL of 50 × ROX reference dye, 2 μL of template DNA (DNA < 80 ng), and 7.8 μL of distilled water (DNase I treated). The qPCR assays were conducted on an Applied Biosystems ViiA7 Real-Time PCR System (ABI, USA). The temperature program for the quantification of ARGs was as follows: initial denaturing at 95 °C for 1 min, followed by 40 cycles of 15 s at 95 °C, annealing temperatures of each gene (Table S2) for 30 s, 72 °C for 30 s, and a final step for a melting curve. The standard curve of each gene was generated by 10-fold dilution of plasmids carrying the target gene, ranging from 10^6 copies to 10^1 copies, with three replicates. The copy number of each ARG and 16S rRNA gene was calculated from the corresponding standard curve using the C_T value of each gene in the qPCR runs. The square of the related coefficient (r^2) of the standard curve ranged from 0.99 to 0.998, and the amplification efficiency ranged from 95% to 110%.