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Prevalence of sulfonamide and tetracycline resistance genes in drinking water treatment plants in the Yangtze River Delta, China

Xueping Guo [a,b], Jing Li [a,b], Fan Yang [a,b], Jie Yang [c], Daqiang Yin [a,b,*]

[a] State Key Laboratory of Pollution Control and Resources Reuse, College of Environmental Science and Engineering, Tongji University, Shanghai 200092, China
[b] Key Laboratory of Yangtze River Water Environment, Ministry of Education, College of Environmental Science and Engineering, Tongji University, Shanghai 200092, China
[c] College of Biological Science and Engineering, Fuzhou University, Fuzhou 350108, China

HIGHLIGHTS

- The presence of 10 ARGs was investigated throughout seven DWTPs in the Yangtze River Delta, China.
- ARGs were more abundant in finished water than in source water in two plants.
- ARG removal efficiencies of advance treatments not superior to conventional ones.
- Fate of ARGs in the same treatment process could vary in different plants.

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ABSTRACT

The occurrence and distribution of antibiotic resistance genes (ARGs) in drinking water treatment plants (DWTPs) and finished water are not well understood, and even less is known about the contribution of each treatment process to resistance gene reduction. The prevalence of ten commonly detected sulfonamide and tetracycline resistance genes, namely, *sul* I, *sul* II, *tet*(C), *tet*(G), *tet*(X), *tet*(A), *tet*(B), *tet*(O), *tet*(M) and *tet*(W) as well as 16S-rRNA genes, were surveyed in seven DWTPs in the Yangtze River Delta, China, with SYBR Green I-based real-time quantitative polymerase chain reaction. All of the investigated ARGs were detected in the source waters of the seven DWTPs, and *sul* I, *sul* II, *tet*(C) and *tet*(G) were the four most abundant ARGs. Total concentrations of ARGs belonging to either the sulfonamide or tetracycline resistance gene class were above 10^5 copies/mL. The effects of a treatment process on ARG removal varied depending on the overall treatment scheme of the DWTP. With combinations of the treatment procedures, however, the copy numbers of resistance genes were reduced effectively, but the proportions of ARGs to bacteria numbers increased in several cases. Among the treatment processes, the biological treatment tanks might serve as reservoirs of ARGs. ARGs were found in finished water of two plants, imposing a potential risk to human health. The results presented in this study not only provide information for the management of antibiotics and ARGs but also facilitate improvement of drinking water quality.

1. Introduction

Antibiotic resistance genes (ARGs), a class of emerging contaminants (Pruden et al., 2006), have attracted considerable concern due to their potential ecotoxicological risks and threat to public health (Allen et al., 2010; Martinez, 2008; Pruden et al., 2006). Although most ARGs carried by fungi and bacteria exist widely in nature, human abuse of antibiotics has favored selection and spread of antibiotic resistance. Since their first reported discovery, ARGs and antibiotic resistance bacteria (ARB) have been detected extensively in natural environments (Daughton and Ternes, 1999), such as surface rivers (Luo et al., 2010, 2011; Munoz-Aguayo et al., 2007), groundwater (Bockelmann et al., 2009) and sediments (Neela et al., 2007), among which aquatic ecosystems are recognized as one of the most important reservoirs for ARB. ARGs present on plasmids, transposons and integrons can be transferred between bacterial cells and species (Alonso et al., 2001; Walsh, 2000) in water, sediments and soil, and even to human commensal pathogens and bacteria (Forsberg et al., 2012). According to a recent report entitled, "Antibiotic Resistance Threats in the United States, 2013" issued by the Centers for Disease Control and Prevention, USA, Gram-negative pathogens are gaining resistance to almost all treatment drugs.

ARGs and ARB in finished drinking water have been investigated in several studies by different approaches, such as culture-dependent methods (Xi et al., 2009), indicator organisms (Scoaris et al., 2008) and qualitative and quantitative molecular techniques (Figueira et al.,

* Corresponding author at: State Key Laboratory of Pollution Control and Resources Reuse, College of Environmental Science and Engineering, Tongji University, Shanghai 200092, China. Tel./fax: +86 21 65982688.
E-mail address: yindq@tongji.edu.cn (D. Yin).

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2011; Storteboom et al., 2010; Xi et al., 2009). For example, *van* A, a gene supposedly carried by biofilm-forming enterococci, was found in the drinking water distribution system even in the absence of enterococci, suggesting possible gene transfer to autochthonous drinking water bacteria (Schwartz et al., 2003). It is noteworthy that the resistant genes could be transferred to human commensal pathogens and bacteria through drinking water (Hunter et al., 2008; Schwartz et al., 2003). In addition, multiply antibiotic resistant bacteria in drinking water were analyzed and found to be selected during treatment processes (Armstrong et al., 1981). Despite accumulating evidence indicating ARG persistence and dispersal in drinking water, knowledge regarding the fate of ARGs during various treatment processes in DWTPs is still lacking, which may be due to low concentrations of ARGs in DWTPs as well as complex influencing parameters. It is likely that the physical, chemical and biological processes adopted in drinking water treatment have specific impacts on ARGs and ARB. For example, effects of chlorination on ARGs can be affected by microbial diversity and interspecies relationships (Berry et al., 2006). Consistently, in an investigation on the disinfection of swine wastewater, ARB cultured on tetracycline-amended media are more susceptible to chlorine (Macauley et al., 2006). Another study (Farkas et al., 2013) demonstrated that biofilm communities in a DWTP might be a reservoir of class 1 integron genes. However, the detailed distribution of ARGs during various treatment processes in DWTPs is seldom reported.

The Yangtze River Delta is China's first economic zone and among the world's largest urban agglomerations. It is also one of the most populated areas in the world. Therefore, the quality of drinking water in this area is of great importance and draws much attention. To ensure the safety of drinking water in this densely inhabited area, various types of drinking water treatment processes are employed in the Yangtze River Delta, including conventional and advanced treatment processes. As a type of advanced treatment technology, biological treatment processes such as bio-pretreatment and O_3-BAC (biological activated carbon technology) are commonly used in the DWTPs carrying abundant bacteria communities, which might be a potential reservoir of ARB. However, little is known whether these bacteria communities and the operation mode of biological treatments have potential influence on the fate, spread and proliferation of ARGs.

In this study, we used real-time quantitative polymerase chain reaction (qPCR) to document the prevalence of tetracycline and sulfonamide resistance genes in various DWTPs in the Yangtze River Delta, China. The objectives of the present study were (i) to quantify the presence of ten ARGs in raw water and treatment stages of seven DWTPs in Yangtze River Delta, China, (ii) to check for the presence of ten ARGs in finished water and (iii) to assess and compare the contribution of conventional treatment and advanced treatment processes to attenuate ARGs from DWTPs. Based on our previous studies on the prevalence of ARGs in the Huangpu River and raw drinking water of Shanghai (Jiang et al., 2013), we chose the following as the target resistance genes in this study: sulfonamide resistance genes *sul* I and *sul* II and tetracycline resistance genes *tet*(C), *tet*(G), *tet*(X), *tet*(A), *tet*(B), *tet*(O), *tet*(M) and *tet*(W). The results presented in this study not only provide information for the management of ARGs but also facilitate the improvement of drinking water quality.

2. Material and methods

2.1. DWTPs and sample collection

Seven DWTPs were chosen in this study; two in Jiaxing, Zhejiang Province, two in Suzhou, Jiangsu Province, one in Wuxi, Jiangsu province, and the other two in Shanghai. Detailed information on the DWTPs, such as location, water supply capacities and years in operation, is shown in Fig. S1 (Supplementary Material). Plant H adopted two separate treatment schemes and thus was considered as two plants here, namely, Plants H1 and H2. These seven DWTPs involved typical treatment schemes in the Yangtze River Delta, the most rapidly developing area of China. Various processes, including biological pretreatment, coagulation, flocculation, sedimentation, ozonation (primary disinfection), sand filtration, BAC and chlorination (secondary disinfection), are adopted. A summarization of the water source and treatment scheme for each plant can be found in Table 1.

Samples were collected from source and finished water of each plant, as well as from selected treatment processes of interest. Three samples were taken from each site, stored in 4 L sterile amber glass bottles and transported to the laboratory on ice in foam boxes.

2.2. DNA extraction

For each sample, 1 to 3 L of water was used for DNA extraction depending on estimated ARG abundance. Water samples were filtered through 0.22 μm mixed cellulose esters membrane filters (Millipore, Australia) to capture bacteria. The membrane filters were quartered and stored at −20 °C in 50 mL centrifuge tubes until DNA extraction. DNA was extracted from the membranes using Water DNA Kit (Omega, USA) according to the manufacturer's protocol and stored at −20 °C until analysis.

2.3. Real-time quantitative PCR

Real-time quantitative PCR (qPCR) of ARGs was conducted with Applied Biosystems 7500 Fast Real-Time PCR System (Life Technology, USA). The 20 μL reaction mixture consisted of 12.3 μL ultrapure water, 2 μL Mg^{2+} (25 mmol/L), 2 μL 10× PCR buffer, 0.5 μL 25 mM dNTPs, 0.5 μL of each forward and reverse primer (10 μM) (Supplementary Material, Table S1), 1 U Taq polymerase, 1 μL SYBR Green (20×) and 1 μL DNA template. The thermocycling parameters were as follows: initial denaturation for 2 min at 95 °C, followed by 40 cycles of denaturation for 10 s at 95 °C, annealing for 30 s at 60 °C and extension for 45 s at 70 °C, with a final extension for 6 min at 70 to 95 °C. Sterile water was applied as the negative control, and the 16S-rRNA was also determined for each sample as the reference gene. To ensure reproducibility, each sample was quantified in triplicate, and three independent samples were analyzed from each sampling site. The mean of these nine measurements is reported with standard deviations.

The copy numbers of the ARGs were calculated using the standard curves generated with recombinant plasmids carrying respective ARGs, and the plasmids were also used as the positive controls in qPCR. Ten-fold serial dilutions of the plasmids from 10^2 to 10^9 copies/μL were tested in triplicate under the conditions described above. For all curves, determination coefficients (R^2) were greater than 0.998, and the related determination parameters are presented in Table S2 (Supplementary Material).

3. Results and discussion

3.1. Occurrence of the sul and tet resistance genes in raw water

Raw water was collected from the inlet of each plant. After qPCR, quantities of *sul* and *tet* resistance genes were normalized to the corresponding 16S-rRNA gene copy numbers, and the obtained relative abundance data are mainly reported in this study. Gene quantities normalized to milliliter of water are also presented as the gene copy numbers to compare absolute concentrations and reduction of ARGs. All 10 ARGs were found in the raw water samples of the seven DWTPs (Figs. 1 and 2). The copy numbers of the *sul* and *tet* class genes ranged from 10^5 to 10^6 copies/mL with 16S-rRNA levels of 10^7 to 10^9 copies/mL (Fig. S2 of Supplementary Material). Greater total amounts of *tet* genes compared to *sul* genes were observed in Plants JS, JG and SW, whereas for Plants H, W and SX, *sul* class genes were more abundant. The obtained results indicate high abundance and detection frequencies of ARGs in water sources in the Yangtze River Delta. Among the detected ARGs, *sul*