Characterizing the antibiotic resistance genes in a river catchment: Influence of anthropogenic activities

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ARTICLE INFO

ABSTRACT

Previous studies on environmental antibiotics resistance genes (ARGs) have focused on the pollution sources such as wastewater treatment plants, aquaculture and livestock farms, *etc.* Few of them had addressed this issue in a regional scale such as river catchment. Hence, the occurrence and abundances of 23 ARGs were investigated in surface water samples collected from 38 sites which located from the river source to estuary of the Beijiang River. Among them, 11 ARGs were frequently detected in this region and 5 ARGs (*sul*I, *sul*II, *tet*B, *tet*C, and *tet*W) were selected for their distribution pattern analysis. The abundances of the selected ARGs were higher in the upstream (8.70×10^6 copies/ng DNA) and downstream areas (3.17×10^6 copies/ng DNA) than those in the midstream areas (1.23×10^6 copies/ng DNA), which was positively correlated to the population density and number of pollution sources. Pollution sources of ARGs along the Beijiang River not only had a great impact on the abundances and diversity, but also on the distribution of specific ARGs in the water samples. Both *sul*I and *sul*II were likely originated from aquaculture farms and animal farms, *tet*W gene was possibly associated with the mining/metal melting industry and the electric waste disposal and *tet*C gene was commonly found in the area with multiple pollution sources. However, the abundance of *tet*B was not particularly related to anthropogenic impacts. These findings highlight the influence of pollution sources and density of population on the distribution and dissemination of ARGs at a regional scale.

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Introduction

Antibiotics have been widely used in medical care, husbandry, aquaculture, and agriculture (Aarestrup et al., 2001). The 25%–75% of the used antibiotics in human or livestock which are excreted in intact form in feces and urine, resulted in the continuous discharge of antibiotics into the environment (Bu et al., 2013; Daughton and Ternes, 1999). The released antibiotics may accelerate the development and dissemination of antibiotic resistance bacteria (ARB) and antibiotic resistance genes (ARGs) in the environment (Alonso et al., 2001). Moreover, ARGs can transfer between different bacterial strains through horizontal gene transfer (HGT) and migrate between connected aquatic systems. The wide spread of ARGs and ARB would increase the risk to human health because of the ineffectiveness of antibiotic for treating infectious bacterial diseases. Therefore, ARGs are

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considered to be the global emerging pollutants (Levy, 1998; Pruden et al., 2006).

With the increasing concern of ARGs, many studies have been conducted to investigate the occurrence of these emerging pollutants in various aquatic environments, including river delta (Jiang et al., 2013; Zheng et al., 2011), estuary (Zheng et al., 2011), groundwater (Chee-Sanford et al., 2001), and water supply reservoir (Huerta et al., 2013; Su et al., 2014). It has also been suggested that anthropogenic activities had significant influence on the occurrence and dissemination of ARGs in aquatic environment (Chen et al., 2013a; Su et al., 2014). Studies had been conducted in many specific contamination sources to reveal their contributions of ARG pollution in aquatic environment, including wastewater treatment plants (WWTPs) (LaPara et al., 2011), hospitals (Rodriguez-Mozaz et al., 2015), aquaculture farms (Gao et al., 2012), and livestock farms (Koike et al., 2007; Zhang et al., 2013b). However, the influence of these anthropogenic sources on the distribution of ARGs in a regional catchment was rarely reported.

The Beijiang River, one of the main tributaries flowing through the Pearl River Delta Region, is the most densely urbanized region in Southern China. Population density, as well as the number and diversity of potential pollution sources of ARGs and ARB varied from source to estuary, which make the Beijiang River a proper object to study the influence of anthropogenic activities on the distribution of ARGs on a regional scale. The objectives of the present study are (1) to investigate the profiles of ARGs along the Beijiang River, (2) to uncover the influence of anthropogenic activities on the distribution of ARGs, and (3) to identify the environmental factors affecting the distribution of ARGs in aquatic environment.

In this study, a total of 23 ARGs in 5 classes (sulfonamides, β-lactams, erythromycins, chloramphenicol, and tetracycline resistance genes) which has been found prevalent in surface water were investigated (Pei et al., 2006; Stoll et al., 2012) and covered the common used antibiotics in medical care and husbandry industry (Aydin et al., 2015; Cheng et al., 2013). For example, genes of sulI, sulII, tetA, tetB, tetC, tetG, tetM, tetO, tetW, and tetX were frequently detected in surface water and drinking water samples (Jiang et al., 2013). Among them, 5 ARGs with high frequency of detections (FODs) were selected for further quantification analysis with regard to their distribution and impacts by the potential pollution sources in the Beijiang River catchment.

1. Materials and methods

1.1. Sample collection

A total of 38 surface water samples were collected using the glass samplers along the Beijiang River from its two separate sources (Sites S1 and S8) to the Pearl River Estuary (Site S43) near the South China Sea. The sampling campaign lasted for 7 days in March, 2013 to investigate the ARG profile in surface water in the dry season of the Beijiang River. The sampling sites were grouped into three categories: upstream area (Sites S1–S12), midstream area (Sites S13–S26), and downstream area (Sites S27–S43) according to the geographic location,

population density and the location of potential ARG sources, including livestock farms, aquaculture farms, WWTPs, hospitals, metal smelting plants and pharmaceutical factories along the Beijiang River (Fig. 1 and Table S1). Generally speaking, the upstream and midstream areas had more feedlot operations, while there were more aquaculture farms and WWTPs located in the downstream area. The population density was higher in the upstream and downstream area than the midstream area as shown in Table S1. Each sampling site was about 10–20 km away from each other.

Surface water samples of 1.5 L (0.5 m below the surface) were collected at each sampling site, stored in the dark at 4°C and transported to laboratory as soon as possible. About 1.5 L water samples were filtered with a 0.22 μm filter membrane using a vacuum pump filter device. The filtrates of the samples were used for antibiotic analysis and the particles remained on the filter membranes were used for DNA extraction and ARG detection.

1.2. Antibiotic analysis

The target antibiotics in water samples were concentrated by solid phase extraction using a HLB column cartridge, and detected using TSQ Quantum Ultra (Thermo Fisher Scientific, Waltham, MA, USA) high performance liquid chromatography with electrospray ionization and tandem mass spectrometry (HPLC-MS/MS) according to a previously reported method (Zou et al., 2011). A total of 10 antibiotics were analyzed, including 4 sulfonamides (sulfadiazine (SDZ), sulfadimidine (SMZ), sulfamethoxazole (SMX), N-sulfanilylacetamide (SAAM)); 2 quinolones (norfloxacin (NOR), ofloxacin (OFL)); 3 macrolides (roxithromycin (RTM), azithromycin (AZM), clarithromycin (CTM)); and chloramphenicol. The chromatographic separation of antibiotics was achieved by a Thermo Hypersil GOLD Dim column (100 mm × 2.1 mm, 1.9 μm particle size). Details of the parameters and implementation of the methods, and the concentration of antibiotics are described in the Supporting Information (Tables S3–S5).

1.3. DNA extraction

Membranes containing the trapped micro-organisms from water samples were extracted using an EZNA Water DNA Kit (OMEGA Bio-tek, USA) according to the protocol. The concentration and quality of the extracted DNA was measured using a Nano Vue Plus Spectrophotometer (Healthcare Bio-Sciences AB, Sweden) and 2% agarose gel electrophoresis.

1.4. Detection of ARGs in water samples

The presence of 23 ARGs was determined by qualitative PCR. The selected ARGs in this study included 2 sulfonamide resistance genes, 3 β-lactam resistance genes, 3 chloramphenicol resistance genes, 2 erythromycin resistance genes and 13 tetracycline resistance genes. The primers used in the qualitative PCR are listed in Table S7. PCR was conducted in a 50 μL solution containing approximately 30 ng sample DNA, 1.5 μL each primer (3 μmol/L), 25 μL 2× PCR Mix (Guangzhou Dongsheng Biotech, China), with 100 mmol/L KCl, 20 mmol/L Tris–HCl, 3 mmol/L $MgCl_2$, 0.4 mmol/L dNTP mixture and 0.1 U/μL Taq polymerase.