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Contamination profiles of antibiotic resistance genes in the sediments at a catchment scale

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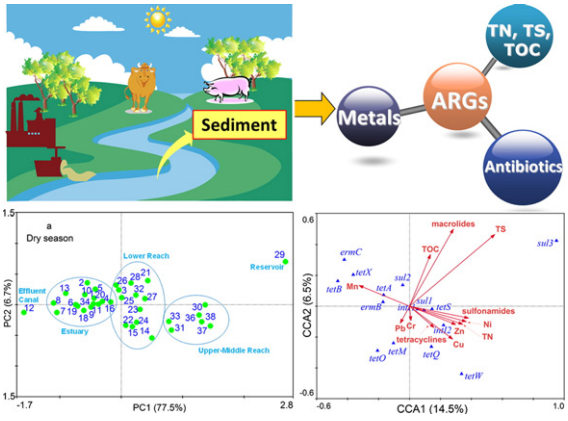
HIGHLIGHTS

- ARGs of tetracycline, sulfonamide, macrolide, and integrons detected in sediment
- *sul2* was the highest resistance gene and the *intI2* was found to be the lowest.
- The distribution of ARGs was correlated to the sediment properties.
- Sediment is a reservoir of ARGs and plays a key role in disseminating ARGs in basin.

GRAPHICAL ABSTRACT



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ABSTRACT

The aim of this study was to investigate the contamination profiles of tetracycline, sulfonamide, and macrolide resistance genes, as well as integrons in sediments of Dongjiang River basin of South China by real time quantitative polymerase chain reaction. *sul2* was the most abundant resistance gene, with the average concentration of 6.97×10^8 copies/g and 1.00×10^8 copies/g in the dry and wet seasons, respectively, followed by *ermF*, *sul3*, *sul1*, *intI1*, *tetA*, *ermB*, *tetX*, *tetM*, *tetQ*, *tetO*, *tetW*, *tetS*, *ermC*, and *tetB*. The abundance of *intI2* gene was the lowest in the sediment samples. Significant correlations existed between the ARGs and sediment properties as well as metals (Cu and Zn) and corresponding antibiotic classes, suggesting that the contamination of ARGs is related to chemical pollution of the sediments in the river basin. Principal component analysis showed distinct groupings of the sampling sites, reflecting that human activities are the key player in the dissemination of ARGs in the catchment environment.

1. Introduction

The extensive application of antibiotics in human, livestock, and agriculture has led to environmental contamination of antibiotic residues,

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antibiotic resistant bacteria (ARB), and antibiotic resistance genes (ARGs), which has become a global public concern (Pruden et al., 2006). According to a World Health Organization (WHO) report, over two million Americans are infected with resistant pathogens each year, and 14 thousand people die consequently (WHO, 2000). As emerging environmental contaminants, ARGs have been broadly detected in diverse environmental compartments, such as hospital wastewater (Durham et al., 2010; Sidjabat et al., 2006; Vinue et al., 2010), waste water treatment plants (Caplin et al., 2008; LaPara et al., 2011; Zhang and Zhang, 2011), chicken farms (Xia et al., 2010), beef farms (Hoyle et al., 2006), pig farms (Xia et al., 2010), dairy farms (Srinivasan et al., 2005), aquaculture farms (Ishida et al., 2010; Tamminen et al., 2011), surface water and sediment (Pei et al., 2006; Storteboom et al., 2010a, 2010b; Yang and Carlson, 2003), drinking water treatment and distribution systems (Faria et al., 2009; Xi et al., 2009), and even food-producing animals (Deckert et al., 2010; Hughes et al., 2010; Schweitzer et al., 2011). ARGs may be transferred from human and animal sources to different environmental compartments at a regional scale, thus threatening human health; therefore, there is a need to understand their contamination profiles at the regional scale and their relationships to land uses and human activities.

ARGs could be disseminated via the water cycle in a catchment. The sediment phase of a river is potentially a reservoir of various contaminants including antibiotics and ARGs. Diverse ARGs have been reported in the sediment phase of rivers in several countries; for instance, tetracycline resistance genes and sulfonamide resistance genes in the USA (Pei et al., 2006; Pruden et al., 2006; Storteboom et al., 2010b) and in China (Luo et al., 2010), extended-spectrum beta-lactamase (ESBL) genes in China (Lu et al., 2010), and quinolone resistance genes in India and Sweden (Kristiansson et al., 2011). It is known that microorganisms can obtain the ARGs in the environment via horizontal gene transfer (HGT), such as plasmids, integrons, and transposons (Andersson and Levin, 1999; Kruse and Sorum, 1994; Pruden et al., 2006). Hence, the diversity and abundance of ARGs in sediments of a river basin are important information for us to understand the dissemination of antibiotic resistance at the catchment scale.

To understand the characteristics and contamination profiles of ARGs at the catchment scale, the Dongjiang River basin in South China was selected as the study area as it includes different land uses and economic development levels from the upper reach to estuary. The Dongjiang River provides the main drinking water source for a receiving population of over 40 million people in several metropolitan cities in the Pearl River Delta region. So far, little information has been known about the influence of human activities on contamination levels of ARGs in the Dongjiang River basin. The objective of this study was to investigate the contamination profiles of antibiotic resistance genes (ARGs) in the sediments of the Dongjiang River basin in both dry and wet seasons by real time quantitative polymerase chain reaction (qPCR), and evaluated the relationships between the spatial distribution of ARGs and the extent of chemical pollution at the catchment scale. Sediment samples were collected from 36 monitoring stations in the river basin from its upper reach to estuary. The ARGs selected in this investigation include tetracycline resistance genes, sulfonamide resistance genes, macrolide resistance genes, and integrons genes, which are related to some commonly used antibiotics. The results from this study can assist better understanding of the diversity, abundance, and dissemination of ARGs at the catchment scale, and their relationship with anthropogenic influence.

2. Materials and methods

2.1. Study sites and sample collection

Sediment samples were collected at 36 stations in the Dongjiang River basin in December 2008 (dry season) and July 2009 (wet season). The sampling sites include 4 sites in the upper reach, 5 sites in the middle reach, 10 sites in the lower reach, and 17 sites in the estuary of the Dongjiang River basin (Fig. 1). It should be noted that sites 17 and 35 out of all 38 monitoring stations in the river basin (Fig. 1) were not accessible during the sampling campaigns of this study. Basic information about water quality parameters, land use, population and livestock for each site, and different reaches are listed in Tables S1 and S2 (Supporting Information). Three sediment samples were aseptically collected from each site with sterile containers, immediately placed on ice, transported back to the laboratory, and stored in −20 °C until processing within a week.

2.2. Sediment characterization

Basic sediment quality parameters including total nitrogen (TN), total sulfur (TS), and total organic carbon (TOC) as well as particle size distribution were characterized and listed in Tables S3 and S4. Total organic carbon (TOC, %) of each sediment sample was determined by using an LECO C230 carbon analyzer (USA) after removal of carbonates with HCl, whereas its particle size distribution was analyzed by using the pipette method (Wang et al., 2011). The total nitrogen (TN) was determined by using the Kjeldahl method and the total sulfur (TS) was determined by using an elemental analyzer (Islam et al., 2004). Antibiotics in the sediment samples were quantified by rapid resolution liquid chromatography-electrospray ionization tandem mass spectrometry with the previous method as described by Zhou et al. (2012). The selected antibiotics included five sulfonamides (sulfamethoxazole, sulfadiazine, sulfapyridine, sulfamethazine, trimethoprim), four tetracyclines (oxytetracycline, chlortetracycline, doxycycline, tetracycline), and three macrolides (erythromycin-H_2O, roxithromycin, oleandomycin). Six metal elements, i.e. chromium (Cr), manganese (Mn), nickel (Ni), copper (Cu), zinc (Zn), and lead (Pb), were determined by inductively coupled plasma-mass spectrometry (ICP-MS, Agilent 7500, Agilent, USA) (Yuan et al., 2004).

2.3. DNA extraction and purification

Sediment samples were freeze-dried, ground with a mortar and sieved through a 100-mesh screen. DNA was extracted from each sediment sample using the PowerSoil DNA Isolation Kit (Mobio, USA). Exactly 0.5 g of each sediment sample from every site was used for DNA extraction. The DNA extraction steps followed the protocol provided by the manufacturer. Then DNA was further purified using the DNA Spin Kit (Tiangen, China) to minimize PCR inhibition.

2.4. ARG quantification

Absolute quantification standard curve method was used to quantify the ARGs in the sediment samples. SYBR Green Real Time QPCR Kit (TOYOBO, Japan) was applied to quantitatively determine the abundance of resistance genes. The specific primers of 16 genes are listed in Table S5. Positive controls consisted of cloned and sequenced PCR amplicons obtained from the sludge of wastewater treatment plants and manures of livestock farms. Both positive and negative controls (Milli-Q water) were included in every run. A total of 40 cycles was applied to improve the chances of product formation from low initial template concentrations. A 20 μL PCR reaction solution was employed: 2 × THUNDERBIRD SYBR® qPCR Mix 10 μL, 0.05 mM each primer 0.08 μL, 50 × ROX reference dye 0.04 μL, template DNA 2 μL (DNA < 80 ng), and distilled water 7.8 μL (DNase I treated). The qPCR assays were run on an Applied Biosystems 7500 Fast Real-Time PCR System (ABI, USA). The temperature program for quantification of ARGs consisted of initial denaturing at 95 °C for 1 min, followed by 40 cycles for 15 s at 95 °C, 55 °C for 30 s, 72 °C for 30 s, and a final step for melting curve. The standard curve was used to calculate the copy number of ARGs, with the square of related coefficient (r^2) of the standard curve > 0.99, and the amplification efficiency ranging from 95% to 110%.