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Antibiotic resistance genes in lakes from middle and lower reaches of the Yangtze River, China: Effect of land use and sediment characteristics

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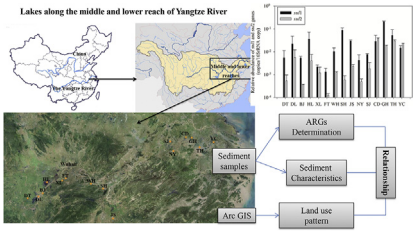
HIGHLIGHTS

- Diversity and abundance of ARGs in 15 lakes along the Yangtze River were shown.
- Redundancy analysis was used to analyze the ARG abundance determined by qPCR.
- The lakes with high proportion of built-up land use had high ARG abundance.
- Sediment characteristics had no significant effect on ARG distribution.

GRAPHICAL ABSTRACT



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ABSTRACT

Freshwater lakes provided an ideal media for the accumulation and propagation of antibiotic resistance genes (ARGs), because they were susceptible to anthropogenic impacts. Land reclamation and urbanization exerted severe anthropogenic impacts on lakes from middle and lower reaches of the Yangtze River, China over the past decades. In this study, 15 lakes in the region were selected to understand the level and variability of ARGs. Proportion of different land use types was applied to display the land reclamation and urbanization around each lake. For sulfonamide resistance (*sul*) genes, *sul*1 had the highest relative abundance in sediments, with maximum 2.11×10^{-1} copies/16SrRNA copy in Gehu Lake. For tetracycline resistance (*tet*) genes, *tet*G had the highest average value of relative abundance (4.74×10^{-3} copies/16SrRNA copy), followed by *tet*B, *tet*A, *tet*Q and *tet*M. Class I integron (*int*I1) played an important role in acquisition and dissemination of *sul*1 and *tet*G. Sediment characteristics (moisture, density, total nitrogen, total carbon, ammonium, and nitrate) were found to have no significant effect on ARG distribution. Taihu Lake and Yangcheng Lake which exhibited high *sul* and *tet* genes had the high proportion of built-up land use.

1. Introduction

Antibiotics have saved millions of lives since their application in 1930s, but the increasing antibiotic resistance genes (ARGs) in bacteria was a growing cause of concern. Pathogens carrying ARGs

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have been becoming resistant to the most commonly prescribed antibiotic treatments, resulting in prolonged illness and greater risk of death (Cosgrove, 2006). The aquatic environment was an important pool for ARGs, because many pollutants from wastewater treatment plants, industrials, hospitals and swine farms finally circulated in water environments and drove the propagation of ARGs (Zhang et al., 2009; Liu et al., 2012; Zhu et al., 2013; Lavilla Lerma et al., 2014). Evidence showed that even subinhibitory concentrations of antibiotics might promote antibiotic resistance (Kümmerer, 2009). Among the aquatic environment, rivers have received the most attention during to its rapid transport of ARGs and obvious identification of pollution source and landscape in different reaches (Storteboom et al., 2010; Pruden et al., 2012; Chen et al., 2013; Rodriguez-Mozaz et al., 2015). However, lakes with long retention time of pollutants having the potential to store and accumulate more ARGs were paid little attention relatively (Czekalski et al., 2015).

China is a country with high human and veterinary antibiotic consumption, and antibiotic resistance has been a serious public health threat in China (Hvistendahl, 2012). Lakes along the middle and lower reaches of Yangtze River were in the rapid economic development region of China. Serious destruction (land reclamation and urbanization) in this region led to changes in lake morphology and pollution in lakes (Yang and Lu, 2014). The constructed hospitals and wastewater treatment plants during the urbanization might be a very important source of ARGs in lakes via receiving discharged effluents. Hence, we assumed that the land use might have significant effect on ARG distribution in lakes. The relative abundance of ARGs in sediment of 15 lakes along the Yangtze River was explored in this study. Eighteen different ARGs (*sul*1, *sul*2, *sul*3, *tet*A, *tet*B, *tet*C, *tet*M, *tet*W, *tet*Q, *tet*O, *tet*G, *qnr*A, *qnr*B, *qnr*D, *qnr*S, *erm*A, *erm*B and *erm*C) were scanned by conventional PCR analysis to give us a profile of ARG pollution in 15 lakes along the Yangtze River. Sediment was selected as the targeted media, because it reflected a long-term pollution status and stored easily for ARGs analysis. Different land use pattern was applied to display the land reclamation and urbanization around each lake. The effect of lake morphology and sediment characteristics on ARG abundance was also investigated.

2. Materials and methods

2.1. Sampling sites and sample collection

Many lakes of China are in the middle and lower reaches of the Yangtze River form a shallow lake group unique in the world. In this study, 15 lakes were selected non-randomly on the basis of ease of access in this region for ARGs analysis including Datong Lake, Bajiao Lake, Dongting Lake, Xiliang Lake, Honghu Lake, Wanghu Lake, Futou Lake, Saihu Lake, Junshan Lake, Nanyi Lake, Shijiu Lake, Gehu Lake, Changdang Lake, Taihu Lake, and Yangcheng Lake. The 15 lakes varying in size and geographic location (Fig. 1 and Table 1) were sampled during the summer from July 30 to August 7, 2012. Two sampling sites were set at each lake. At each site, three replicate surface sediments were randomly collected within an approximately 50 m^2 area from a boat using a Peterson dredge and then mixed and homogenized to form a composite sample. Then, 0.5 kg of sediment was collected, placed in a sealed plastic bag and stored at approximately 4 °C in a refrigerator until returned to the laboratory. After that, the samples were transported to the laboratory and stored at −80 °C prior to analysis.

2.2. DNA extraction and assays for detection of ARGs

2.2.1. DNA extraction and conventional PCR assay for ARGs

The sediment samples were lyophilized and ground. Then, exactly 0.5 g of sample was used to extract DNA by FastDNA Spin Kit for Soil (QBiogene, Carlsbad, CA) according to the protocol provided by the manufacturer. After that, Geneclean Spin Kit (QBiogene, Carlsbad, CA) was applied to purify the DNA to minimize PCR inhibition. Finally, spectrophotometer analysis (NanoDrop ND-2000c, Thermo) and 1.5% agarose gel electrophoresis were used to assess the quality and the concentration of DNA.

All conventional PCR assays were performed in a 25 μl volume reaction by 2 × Utaq PCR MasterMix (Beijing Zoman Biotechnology Co., Ltd.) according to the protocol provided by the manufacturer. Qualitative analysis of 18 different ARGs (*sul*1, *sul*2, *sul*3, *tet*A, *tet*B, *tet*C, *tet*M, *tet*W, *tet*Q, *tet*O, *tet*G, *qnr*A, *qnr*B, *qnr*D, *qnr*S, *erm*A, *erm*B and *erm*C), class I integron (*int*I1) and bacterial 16SrRNA gene fragments were analyzed by agarose gel electrophoresis using the published primers (Aminov et al., 2001; Ng et al., 2001; Cummings et al., 2010; Luo et al., 2010; Gaze et al., 2011; Ji et al., 2012; Chen and Zhang, 2013; Mao et al., 2015). The PCR products were randomly selected for sequencing and blast in Antibiotic Resistance Genes Database (ARDB, http://ardb.cbcb.umd.edu/) to avoid bias in the analysis.

2.2.2. qPCR assays for ARGs

The qPCR reactions were performed in a 20 μL reaction mixture according to its protocol provided by the manufacturer. The experiment was carried out in 96 well plated in a 7500 Fast Real-Time PCR system (Applied Biosystems, USA). Standard curves were prepared from plasmids with targeted genes and constructed from serial 10-fold dilutions of plasmids containing the respective gene in a range of 10^8 to 10^2 gene copies. The R^2 values for all standard curves were all higher than 0.99. The efficiency of our reactions ranged from 96% to 115%.

2.3. Sediment characteristics

Six sediment characteristics (moisture, density, total nitrogen, total carbon, ammonium and nitrate) in Table S1 were detected according to the procedures described in our previous study (Liu et al., 2015). Briefly, sediment moisture was measured gravimetrically (24 h at 105 °C) from 30 g sediment samples. Total nitrogen (TN) was measured using the Kjeldahl method after digesting samples in a digestor using a sulfuric acid/mercuricoxide catalyst. Total carbon (TC) content of air-dried samples was analyzed by a TOC analyzer (Vario TOC cube, Elementar, Germany). Nitrate and ammonium were extracted from sediments with 2 M KCl and determined using a continuous flow analyzer (Skalar, the Netherlands).

2.4. Watershed land use pattern

The watershed land use was calculated using ArcGIS 10 software (ESRI, Redlands, California, USA) according to the methods described in our previous study (Liu et al., 2015). The data were interpreted from recent Landsat TM images and obtained from the Data Sharing Infrastructure of Earth System Science in China (http://www.geodata.cn/). Four main types of land use were calculated: (1) vegetation, including forest and grassland; (2) agriculture, including dry land and paddy fields; (3) built-up land, including urban areas, rural settlements and others such as industrial areas, roads, and airports; and (4) water bodies, including lakes, rivers, streams, reservoirs, ponds, and wetlands.