PRELIMINARY STUDIES ON THE POLLUTION LEVELS OF ANTIBIOTIOC RESISTANCE GENES IN LOWER REACHES OF THE YANGTZE RIVER, CHINA

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Introduction

Antibiotics and corresponding resistance genes have been considered as emerging pollutants worldwide[1]. A major concern from antibiotic contamination of the environment is the rapid and increasing number of antibiotic resistance genes (ARGs) found in bacteria. Large amounts of antibiotics and their metabolites are potentially released into different compartments of the environment, which are regarded as the most important factor for the evolution and selection of antibiotic resistance in bacterial pathogens[2]. ARGs can be transferred between nonpathogens, pathogens, and even distantly related organisms, such as Gram-positive and Gram-negative bacteria, through horizontal gene transfer (HGT)[3]. Therefore, antibiotic resistance genes are now considered pollutants.The prevalence and persistence of antibiotic resistance in bacterial pathogens have become an emerging threat to public health, which is raising considerable concern[4]. Tetracycline and sulfonamide are two important classes of antibiotics widely used as prophylactic and therapeutic medication for treating human and animal diseases and benefiting agricultural productivity, which were frequentlydetected in different ranges of concentration both in liquid (wastewater,surface water, groundwater and even drinking water) and solid (sludge, soil and sediment) environmental media[5-8]. In this study, real-time quantitative PCR (RT-PCR) were used for detecting occurrence of 8 antibiotic resistance gene[sul (I), sul (II), sul (III), sul (A), tet (M), tet(O), tet (W), tet (Q)] in water samples collected from the lower reaches of the Yangtze River,China.

Materials and methods

Samples were collected during three sampling events in the lower reaches of the Yangtze River between August and September 2012(Fig 1). Each aqueous samples were filtered through 0.45 μm nitrocellulose membrane (MF-Millipore™, Billerica, MA, USA). Total DNA was extracted using a Fast DNA Spin kit (Sangon Biotech, China) according to the manufacturer's instructions. The concentration and quality of the extracted DNA was determined by spectrophotometer analysis and agarose gel electrophoresis. The final DNA extracts were stored at −20 °C prior to real-time PCR analysis, which is used to study the existence and abundance level of eight representative antibiotic resistance genes in the water. To ensure reproducibility, duplicate PCR reactions were performed for each sample. Sterile water was used as the negative control in every run. Target genes and 16S rRNA were quantified in triplicate using a Real-time PCR analyzer 7500 (ABI, America) and the fluorescent dye SYBR-Green I.

Fig 1 Sampling locations

Results and discussion

The analytical figures of the proposed method were determined and summarized in Table 1 to evaluate the performance of the method. The standard curves showed a satisfactory linearity with the correlation coefficients (R^2) of 0.995 to 0.999. The proposed method was validated and applied successfully for the determination of ARGs in real water samples.

Tab 1 Standard Application Curve of ARGs and 16S-rRNA

Target genes	Slope	Correlation Coefficient R^2	Amplification Efficiency
Reference Genes	-2.644	0.999	38.86%
sul (I)	-3.97	0.995	78.60%
sul (II)	-3.549	0.995	91.32%
sul(III)	-2.682	0.999	136.01%
sul (A)	-2.651	0.999	138.34%
tet(M)	-2.796	0.998	127.82%
tet(O)	-2.877	0.999	122.643%
tet (W)	-2.822	0.998	126.098%
tet (Q)	-2.758	0.999	130.487%

The results show that the eight resistance genes are detected in all samples(Fig 2,Fig 3).In the collected samples, both the absolute copies and the relative abundances of sulfonamide ARGs are higher than those of tetracycline ARGs. It suggests that the sulfonamide resistance gene is dominant in the lower reaches of the Yangtze River.The highest relative abundance of sulfonamide ARGs is sul (I), and tet (O) is the highest among tetracycline ARGs, with the absolute copies$10^{-0.99}$-$10^{-1.10}$and $10^{-1.31}$-$10^{-1.40}$ respectively.

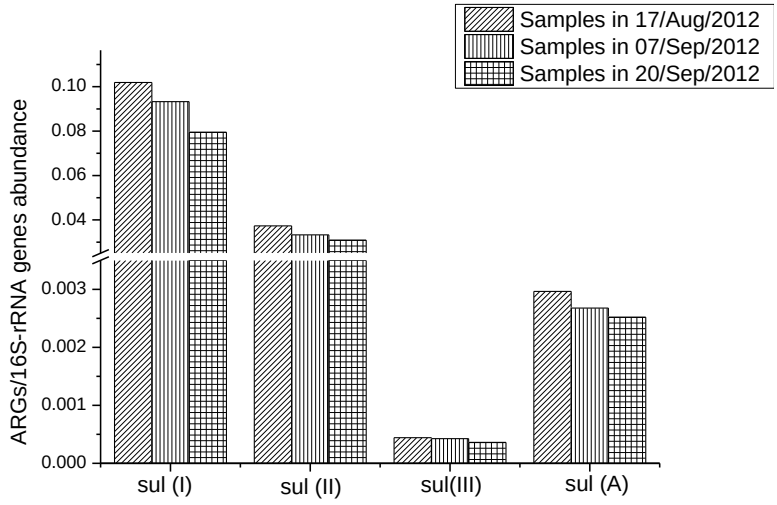

Fig 2 Relative abundance of sulfonamide resistance genes in water samples

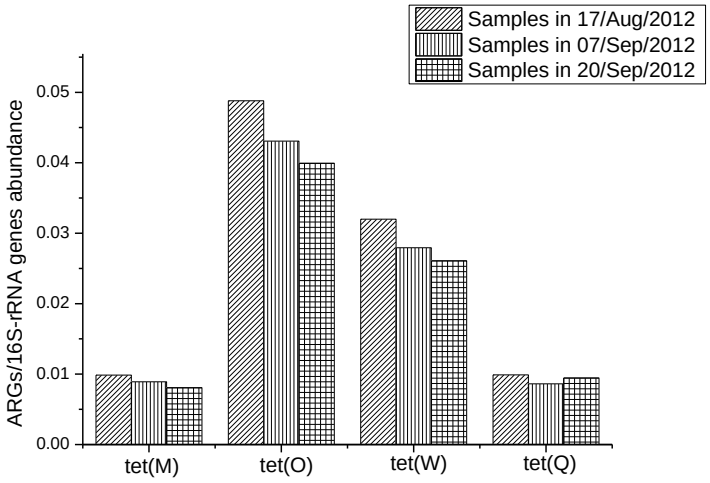

Fig 3 Relative abundance of tetracycline resistance genes in water samples

The Yangtze River is the largest river in China, known as the typical representative of biodiversity conservation.It is an extremely important shipping artery and provides an important part of the raw drinking water for China.With economic and population growth in recent decades, water quality degradation and frequent occurrence of antibiotic contamination have become ubiquitous in the Yangtze River Basin. this study demonstrates that the Yangtze River has been a reservoir of ARGs and antibiotic-resistant bacteria. The presence of ARGs in the Yangtze River would be an threat to quatic food and, consequently,public health through the food chain.Moreover, its unique geographic position posed potential ARG contamination risks to the Yellow Sea.

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