Marine Pollution Bulletin 107 (2016) 245–250

Contents lists available at ScienceDirect

Marine Pollution Bulletin

journal homepage: www.elsevier.com/locate/marpolbul

Occurrence and distribution of antibiotic resistance genes in the coastal area of the Bohai Bay, China

Zhi-Guang Niu [a,b], Kai Zhang [a], Ying Zhang [c,*]

[a] School of Environmental Science and Engineering, Tianjin University, Tianjin 300072, People's Republic of China
[b] School of Marine Science and Technology, Tianjin University, Tianjin 300072, People's Republic of China
[c] MOE Key Laboratory of Pollution Processes and Environmental Criteria, College of Environmental Science and Engineering, Nankai University, Tianjin 300071, People's Republic of China

ARTICLE INFO

Article history:
Received 9 December 2015
Received in revised form 21 March 2016
Accepted 30 March 2016
Available online 20 April 2016

Keywords:
Antibiotic resistance
Bohai Bay
Antibiotic
Correlation

ABSTRACT

Considering the abuse of antibiotics worldwide, we investigated the abundance of three classes of antibiotic resistance genes (ARGs) and the concentrations of corresponding antibiotics in water and sediments of Bohai Bay. The results showed that *sulI* and *sulII* were detected in all samples, and their abundance range was 10^{-5}–10^{-2}/16S gene copies. The abundance of *tetM* and *ermB* were relatively higher than the other genes of *tet*-ARGs and *erm*-ARGs. Sulfonamides were the most prevalent antibiotics, and the concentrations of antibiotic in sediments were higher than those in water. The correlation analysis revealed that antibiotics had pertinence with corresponding ARGs, indicating that antibiotics play an important role in the creation and transfer of ARGs. The results of regression analysis indicated that the propagation and maintenance of *sulI* and *sulII* were facilitated by class I integrons.

1. Introduction

The abuse of antibiotics in human activities, such as animal husbandry and fishery, has led to the increase of antibiotic resistance genes (ARGs) in recent years (Song et al., 2004; Hsu et al., 2010). The resistance gene can be transferred from nonpathogens to pathogens, which reduce susceptibility of pathogens to antibiotics in medical treatment and threatens human health (Pruden et al., 2006; Ji et al., 2012). The "superbug" and "poison cucumber" caused by ARGs have attracted considerable concern. Scientists defined ARGs as a class of emerging contaminants, and studies on the global spread of ARGs are still emerging (Allen et al., 2010; Laxminarayan et al., 2013). The results showed that ARGs can be widely transferred among bacterial species by horizontal transfer in environment, and integrons, an ancient and common feature of bacterial genomes (Gillings et al., 2014), are considered as the key players in horizontal transfer of resistance genes (Rowe-Magnus and Mazel, 2002). Among all the reported ARGs, tetracycline ARGs, sulfonamide ARGs, and macrolide ARGs are observed commonly in the environment (Jiang et al., 2013; Gao et al., 2012a, 2012b; Luo et al., 2011), and the corresponding antibiotic usage patterns were considered to be an important factor in the generation and propagation of ARGs (Kristiansson et al., 2011).

To date, studies on ARGs have mainly focused on natural water bodies, drinking water, soil, livestock farms, and clinical settings (Ji et al., 2012; Xu et al., 2015; Shi et al., 2013; Edelsberg et al., 2014). However, although the ARGs could be transferred via estuaries to sea and induce global pollution, reports on ARG pollution in the coastal area are very few, and these studies mainly discussed the distribution and abundance of *sul* genes (Na et al., 2014). Other classes of ARGs, such as *erm* and *tet* genes, were rarely discussed in marine environment.

Bohai Bay is the largest semienclosed bay located in the northeast part of China. There are more than 100 drains along the coast, and 75% of them discharge pollutants beyond acceptable limits (Zou et al., 2009). Wastewater from Tianjin and Beijing is discharged into Bohai Bay, which causes serious environmental pollution in the region. Many studies proved the widespread occurrence of pollutants in Bohai Bay (Wan et al., 2005; Wei et al., 2008; Gao and Chen, 2012). However, no comprehensive regional field study has characterized the concentrations of antibiotic residues and the amounts of ARGs in the area.

In this study, we analyzed the occurrence and distribution of seven tetracycline genes (two efflux pump genes (*tetB* and *tetL*), four ribosomal protection protein (RPP) genes (*tetM*, *tetO*, *tetQ*, and *tetW*), and one enzymatic modification gene (*tetX*)), two sulfanilamide genes (*sulI* and *sulII*), two macrolide genes (*ermB* and *ermC*), one genetic element (class I integron (*intI*)) and corresponding antibiotics in Bohai Bay. The objectives of this study were to (1) quantify the presence of 10 ARGs in water and sediments of Bohai Bay, (2) determine the

* Corresponding author.
E-mail address: zhangying@nankai.edu.cn (Y. Zhang).

http://dx.doi.org/10.1016/j.marpolbul.2016.03.064
0025-326X/

relationship between ARGs and corresponding antibiotics in marine environment, and (3) assess the role of class I integrons in the transfer of ARGs in marine environment.

2. Materials and methods

2.1. Sampling sites

A total of seven sampling sites were selected in this study, and one surface water sample and one sediment sample were collected from each site (Fig. 1). Sites 1 and 5 (S1 and S5) are the estuary of Duliujian River and Beitang River, respectively, which transfer a large amount of pollutants into Bohai. Site 7 (S7) is located in the aquaculture area. Site 2 (S2) is situated near the tourism area. Sites 3, 4, and 6 (S3, S4, and S6) are present in the areas that are not influenced by human activities. Samples were collected on 12 July 2015.

2.2. Sample collection and store

The upper 0.5 m of the surface water was collected and stored in a 1-L sterile amber glass bottle. The upper 5-cm layer of the sediment samples was collected and stored in self-sealing bags. However, sediment sample was not collocted in S6. All of the samples were placed in an ice bath and transported to the laboratory within 8 h. Before DNA extraction, the water and sediment samples were stored in a laboratory at 4 and −20 °C, respectively.

2.3. Analysis of antibiotic concentration

2.3.1. Pretreatment

The modified pretreatment method of water and sediment samples was conducted in this study (Luo et al., 2010). Briefly, approximately 5 g of lyophilized sediment samples was processed with 30 mL of extraction buffer thrice and the supernatants were diluted to 200 mL by adding pure water. The supernatants of sediment extraction samples were passed through Strata strong anion exchanger (SAX) cartridges, followed by extraction with Oasis hydrophilic–lipophilic balance (HLB) to avoid interference from dissolved organic matter, and the samples containing antibiotics were loaded on HLB cartridges. The eluate from HLB was evaporated in a gentle nitrogen stream. The initial mobile phase (~0.89 mL) was used to increase the final sample volume to 1 mL, which was stored at −18 °C until high-performance liquid chromatography–mass spectrometry (HPLC–MS/MS) analysis was performed. Water samples (200–500 mL) were filtered through 0.45-μm glass fiber filters and then adjusted to pH 5 by adding citrate buffer. The subsequent pretreatment process was similar to that of the supernatants of sediment extraction samples.

2.3.2. Analysis

A total of seven antibiotics, oxytetracycline (OTC), tetracycline (TC), sulfadiazine (SDZ), sulfamethazine (SMZ), sulfamethoxazole (SMX), erythromycin (ETM), and roxithromycin (ROX), were detected with an Alliance HPLC (Waters 2695) equipped with a C18 reverse-phase column and Waters Micromass Quattro Micro™ detector with electrospray ionization (ESI).

The quantification curves of antibiotics yielded good correlation coefficients ($R^2 \geq 0.985$). The detection limit for each antibiotic was defined as the concentration corresponding to the signal-to-noise ratio of 3. Details about analysis of antibiotics are shown in the Supplemental material (Table S1).

2.4. Microbiological analyses

The water samples were filtered through 10-μm membranes to remove impurities and 1000 mL of water samples was filtered through a 0.22-μm membrane (Millipore, USA) (Yin et al., 2013). Then, the membrane was cut into small pieces and stored at −20 °C. DNA was extracted from the cut membrane using the Water DNA kit (Omega, USA) according to the manufacturer's protocol. Surface sediment was freeze-dried before DNA extraction, and then DNA was extracted from 0.25 g of samples using the Soil DNA kit (Omega, USA). Qualitative analysis of DNA extracts was conducted with agarose gel electrophoresis (Gel Doc XR+, Bio-RAD, USA). DNA extracts were maintained at −20 °C before polymerase chain reaction (PCR) analysis.

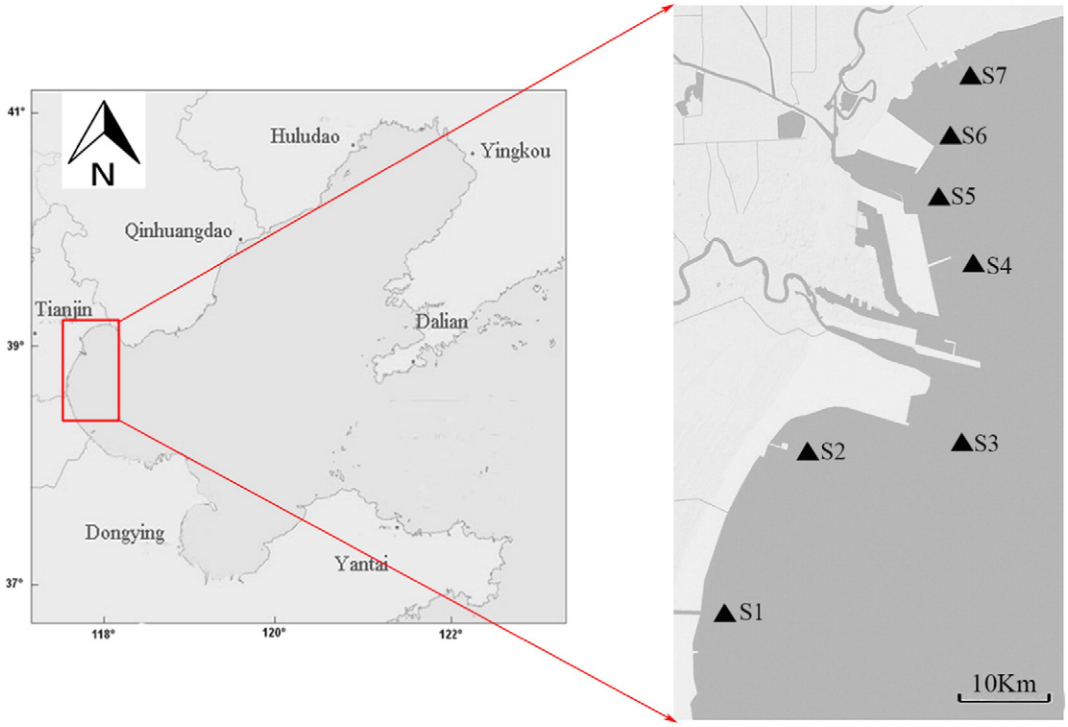

Fig. 1. Sample locations.