



## Occurrences and distribution of sulfonamide and tetracycline resistance genes in the Yangtze River Estuary and nearby coastal area



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### ABSTRACT

The role of highly impacted estuaries needs to be examined with respect to the spread of antibiotic resistance genes in the environment. In the present study, sulfonamide resistance (*sul*), tetracycline resistance (*tet*) and class I integron (*int1*) genes were ubiquitous in the sediments of the Yangtze Estuary (YE) and nearby coastal area, and exhibited a declining trend from the inner estuary to the coast. Good relationships were only observed between *int1* and *sul1* genes, implying that *int1* gene is essential to the proliferation of *sul1* gene. A non-significant correlation between *int1* and 16S rRNA genes indicated that the *int1* gene came from pollution sources of ARGs instead of being intrinsic in environmental bacterial populations. Sulfonamides were rarely detected in the sediments of this region, so could not result in the production of *sul* genes in the local environment.

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### 1. Introduction

Antibiotic resistance genes (ARGs) have been identified as a newly emerging contaminant (Ashbolt et al., 2013; Baquero, 2012; Pruden et al., 2013). Although health risks of ARG pollution have not been accurately assessed (Ashbolt et al., 2013; Pruden et al., 2013), increasing body of evidence demonstrated that ARGs can be exchanged between environmental bacteria and human pathogens via horizontal gene transfer (HGT), or *vice versa* (Stecher et al., 2013; Willems et al., 2011). ARGs in environment bacteria are often identical to those carried by a diverse lineage of clinical pathogens (Forsberg et al., 2012). The evolution of pathogens with multiple antibiotic resistances poses a serious threat to the public health, such as a longer hospitalization period and treatment duration, and treatment failures of infectious disease (Chen and Huang, 2013; Pond et al., 2014).

The aquatic environments are well recognized as one of reservoirs or sinks of ARGs, and their importance is established for the spread and dissemination of ARGs in the environment (Zhang et al., 2009b). ARGs originally occur in the natural environment as a mean of competing or

combating for limited nutrients between microorganisms in terms of species or community (D'Costa et al., 2011; Toth et al., 2010). Nevertheless, ARGs often become abundant in the human-impacted settings with elevated selective stress caused by various pollutants (Modi et al., 2013; Qiu et al., 2012), especially antibiotic use in clinic therapeutic and husbandry (Barraud et al., 2013; Zhu et al., 2013). Consequently, massive amounts of both antibiotic chemicals and antibiotic resistant bacteria from typical anthropogenic sources, e.g., sewage and wastewater treatment plants (Yang et al., 2012), and pharmaceutical manufacturing operations (Khan et al., 2013), are released into nearby aquatic environments. A representative trait of ARGs is that they can be horizontally transferred between microbes using mobile genetic elements (MGEs) as the carriers, e.g., conjugative plasmids and transposons. Integrons associated with these MGEs were initially found to be related to antibiotic resistance (Stokes and Hall, 1989). Integrase-catalyzed recombination leads to the insertion or excision of gene cassettes at certain sites (e.g., *attI* and *attC*), and ARGs resided in these gene cassettes are highly related to a large variety of antibiotics including aminoglycosides,  $\beta$ -lactams chloramphenicol, etc. (Partridge et al., 2009). Until now, integrons have been found in approximately 9% of sequenced bacterial genomes (Labbate et al., 2009), as well as carried by some MGEs (Hall and Collis, 1995; Partridge et al., 2009). Acting as an important gene-acquiring mode, integrons facilitate horizontal gene transfer (HGT) of ARGs between microbes under selective pressure (Di Conza and Gutkind, 2010;

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Gaze et al., 2011; Gootz, 2010), e.g., the *sul1* gene is found in the conserved region of the class 1 integron (*int1*) sequence (Bennett, 2008; Nigro et al., 2013). *Int1* genes are the most abundant gene capture and transmission system in clinical and environmental isolates (Partridge et al., 2009; Shah et al., 2012; Yan et al., 2010), and their abundance and structures were greatly influenced by anthropogenic contamination (Wright et al., 2008).

The Yangtze delta is one of important industrial and economic centers in China, with a high population of more than 25 million. Intensive human activities and operation in this region lead to a huge input of various pollutants into the YE and nearby coastal area, including antibiotics (Shi et al., 2014b; Yan et al., 2013), polycyclic aromatic hydrocarbons (Yang et al., 2008), polychlorinated biphenyls (Zhang et al., 2011), trace metals (An et al., 2009), posing potential environmental risk to the local ecosystem. Although a few studies have been conducted on characterizing typical pollution sources of ARGs in the Yangtze Delta (e.g., drinking water treatment plants) (Guo et al., 2014; Jiang et al., 2013), limited information on the abundance of ARGs and resistance determinants (e.g., *int1* gene) in ambient aquatic environment is available.

Sulfonamides and tetracyclines are widely used in human therapeutics and husbandry, as well as in aquaculture (Tolls, 2001). The usages of sulfonamides and tetracyclines in China in 2013 were estimated to be 7890 and 6950 tons, respectively (Zhang et al., 2015). The objective of this study was to investigate the occurrence and spatial distribution of tetracycline and sulfonamide resistance genes and *int1* gene in the sediments of the YE and adjacent coastal area, which would provide baseline data for source identification and pollution controls of ARGs in this region.

## 2. Materials and methods

### 2.1. Chemicals and standards

Analytical antibiotic standards were purchased from Sigma-Aldrich (St. Louis, MO, USA) for sulfadiazine (SDZ), sulfamethazine (SMZ), sulfamethoxazole (SMX), and sulfacetamide (SMMX) tetracycline (TC).  $^{13}\text{C}_3$ -caffeine was purchased from Cambridge Isotope Labs (1 mg/mL in methanol, USA). Methanol (MeOH) and acetonitrile (ACN) were obtained from Merck (Darmstadt, Germany). Milli-Q water was prepared with a Milli-Q water purification system (Millipore, USA). Stock solutions of antibiotics (100 mg/L) were prepared in the methanol, and were stored in dark at  $-20\text{ }^\circ\text{C}$ . Working solutions were freshly prepared daily for analysis.

### 2.2. Study areas and sample collection

Sediment samples were collected from 13 strategic sites in the YE and nearby coastal area in July 2013, as shown in Fig. 1. The study area under investigation has been highly impacted by rapid urbanization and industrialization, as well as by intensive human activities. Four sampling sites (YZ1–YZ4) were located at the YE area. In the coastal area, four samples were collected from the north of the YE near the coast of Jiangsu province, two samples from the outer part of the YE, and three samples from the Hongzhou Bay in the south of the YE. Surface sediments (200 g) were collected from the top 10-cm layer using a grab sampler. The sediments were immediately mixed well and then frozen at  $-20\text{ }^\circ\text{C}$ . All containers and tools were sterilized prior to use.

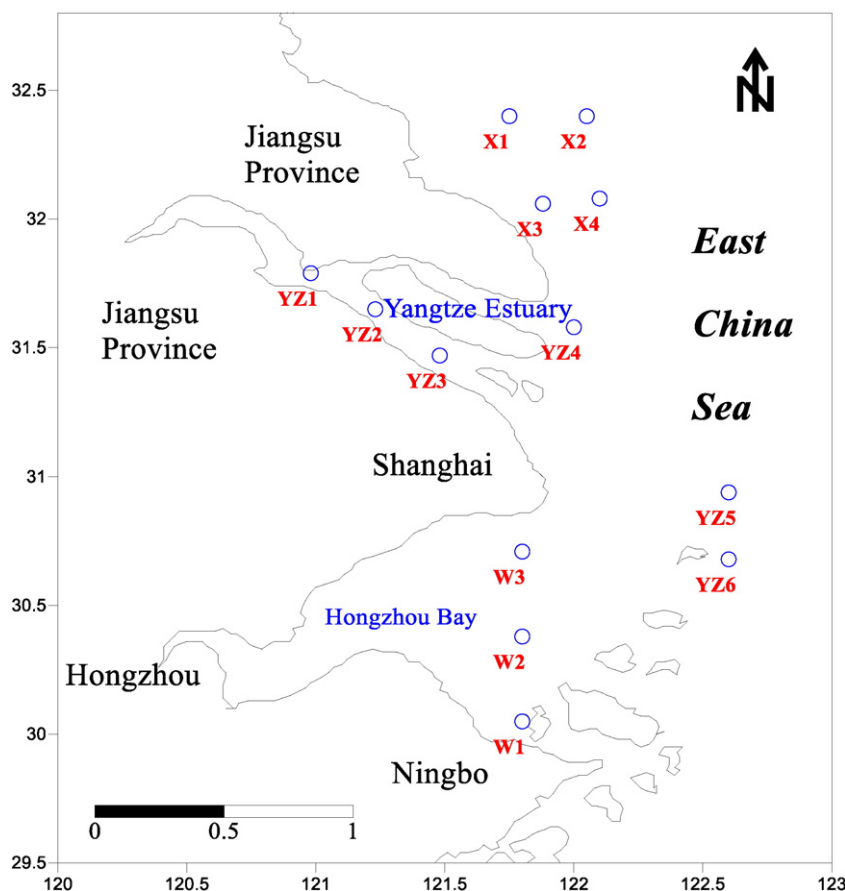


Fig. 1. Sampling sites in the YE and nearby coastal area.

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