# Fate of sulfonamide resistance genes in estuary environment and effect of anthropogenic activities

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## HIGHLIGHTS

- Trends in sul-ARGs were investigated in estuaries under different anthropogenic effect.
- Distribution of sul-ARGs did not correlate with the sulfonamides levels in estuary.
- Significant reduction of sul-ARGs abundances were observed in estuary.
- Fate of sul-ARGs were affected by ambient pressures from non-antibiotic contaminants.

## ABSTRACT

With the exacerbating problem of antibiotic resistance, antibiotic resistance genes (ARGs) as emerging contaminants are found at elevated levels in inland aquatic environments, especially in regions of intensive agricultural and urban activity. However, little quantitative data exist on the migration and attenuation of ARGs in estuary ecosystem, which is central to predicting their fate after release into marine environment. Moreover, the relevance of multiple chemical contaminants and water quality constituents should be understood to amplify and attenuate antibiotic resistance levels. To determine the prevalence and examine the fate of sulfonamide ARGs (*sul*-ARGs) in two estuaries under different effects of anthropogenic activities, we analyzed the *sul*-ARGs (*sul1*, *sul2*, and *sul3*), class 1 integrons (*int1*), and bacterial biomass in surface water samples from Daliaohe and Liaohe river estuaries. We also evaluated five types of antibiotics, heavy metals, and various bulk water quality constituents. Results showed that *sul*-ARGs were widespread in Daliaohe and Liaohe river estuaries, but the distribution did not correlate with the concentration of sulfonamides. Significant reduction in the abundance of *sul*-ARGs was also observed with increased salinity. Nevertheless, the trend in the change of concentrations of *sul*-ARGs was different in the two estuaries. Statistical analysis of the results indicated that several metals were significantly and positively correlated with *sul*-ARGs. Pearson's correlation coefficients were higher than those determined between antibiotic residues and *sul*-ARGs. Furthermore, the relative abundance of *sul*-ARGs was significantly and positively correlated with the relative abundance of *int1* which suggested that the propagation of *sul*-ARGs was facilitated by class 1 integrons in estuaries.

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## 1. Introduction

Antibiotics are used in human medicine and stockbreeding operations to promote infectious disease therapy and growth (Cabello, 2006; Halling-Sørensen et al., 1998; Martinez, 2009). However, a significant fraction of the antibiotics fed to animals cannot be ingested; furthermore, these antibiotics and their metabolites are released unaltered through various approaches in different compartments of the environment (Huang et al., 2011; Hamscher et al., 2002). As a direct consequence of high levels of antibiotics and metabolite residues in the environment, the evolution and selection of antibiotic resistance genes (ARGs) are promoted (Hu et al., 2008). Once present in the environment, ARGs can be widely transferred among bacterial species, even to microorganisms in humans and other animals; as such, ARGs can potentially become prevalent in the environment and pose pre-eminent environmental challenges (Kobayashi et al., 2007; Forsberg et al., 2002; Agerso and Sandvang, 2005). However, limited information is available regarding the environmental behavior and ecological effects of ARGs compared with the cognition of environmental issues caused by pesticides, polycyclic aromatic hydrocarbons, and polychlorinated biphenyls. Thus, a systematic research on ARGs in the environment should be conducted.

Studies in Europe, U.S., and China have focused on the detection of multiple varieties of antibiotic compounds and corresponding resistance genes in samples collected from rivers, irrigation ditches, dairy lagoons, and wastewater treatment plants, which were most susceptible to agricultural and urban activities (X. Zhang et al., 2009; Schwartz et al., 2003; Chee-Sanford et al., 2009; LaPara et al., 2011). The correlation of antibiotics with ARGs had been further discussed (Gao et al., 2012; David et al., 2011). To date, sulfonamide ARGs and tetracycline ARGs are commonly observed in aquatic environments (Goldstein et al., 2001; Pruden et al., 2006; Lei et al., 2013). Research had also shown that the presence and propagation of antibiotic resistance determinants were attributed to the selection pressure from antibiotics; the effect of antibiotic exposure on ARGs depended on concentration (Luo et al., 2010; Ghazanfar et al., 2013; Lei et al., 2013). However, few studies have been conducted to clarify their disappearance kinetics or fate mechanisms after release into the receiving estuary environment, such as migration pattern of ARGs from estuary to marine environment and influence of co-contaminating antibiotics to retention of ARGs in situ. Estuary ecosystem is the ecotone between river and marine environment, which exhibits unique physical and chemical properties and the strongest continental–oceanic interaction. In addition, the antibiotic concentration detected in estuaries is lower than that in adjacent rivers, urban sewage, and other inland aquatic environments at all times. Thus, the fate of ARGs in estuary ecosystem may differ with that in inland aquatic environment.

Relatively limited information is also available regarding the effect of antibiotic exposure to multiple non-antibiotic constituents (e.g., heavy metals, pH, and nutrients) in the same environment. The development and spread of antibiotic resistance only resulted from an evolutionary process. Non-antibiotic contaminant pressures can select bacteria with an elevated mutation rate (hypermutators or mutators) along the lines of antibiotic pressure, thereby indirectly selecting genes with increased probability of resistance to antibiotics (Taddei et al., 1997). Other mechanisms showed that non-correspondence contaminants influence the fate of ARGs by means of co-selection (Chapman, 2003), which included co-resistance (different resistance determinants present on the same genetic element) and cross-resistance (same genetic determinant responsible for resistance to antibiotics and other contaminants). Thus, research on the effect of non-correspondence contaminants not only contributes to understanding of the presence and propagation of ARGs but also reassesses the potential risk of chemical contaminants from the perspective of fitness selection.

The present study aimed to (1) clarify the fate of sulfonamide resistance genes (*sul1*, *sul2*, and *sul3*) in two estuaries under the effects of different anthropogenic activities and (2) investigate the factors influencing the maintenance and propagation of ARGs in the estuaries. To achieve these objectives, we characterized the occurrence and distribution of the sulfonamide resistance genes (*sul1*, *sul2*, and *sul3*) in Daliaohe and Liaohe river estuaries. In addition, we assessed the pollution levels via three aspects: five types of antibiotics, eleven typical heavy metals, and eutrophication index (EI). We also discussed the correlations of sulfonamide resistance genes with antibiotic residues, multiple heavy metal contaminants, and water quality constituents. Furthermore, this study provided indirect field evidence of the selective pressure of ambient factors on associated ARGs.

## 2. Experimental section

### 2.1. Sampling strategy

Liaohe River and Daliaohe River water systems are located between 40° 31′ N to 45° 17′ N and 116° 54′ E to 125° 32′ E in northeastern China. Both water systems enter Liaodong Bay of Bohai Sea via Liaohe River and Daliaohe River estuaries, respectively. Nevertheless, the difference in pollution level between the two estuaries is obvious. Liaohe River ? estuary, as the national natural reserve, is under relatively few <?A3B2 show influences of concentrated anthropogenic activities. By contrast, the midstream and downstream areas of the Daliaohe River system are important industrial bases where oil, chemical, medicine, and steel factories in the northeast part of China are located and distributed in large industrial cities, such as Shenyang, Benxi, Anshan, and Yingkou. Approximately 2074 million tons of industrial and domestic wastewater is discharged annually into the Daliaohe River catchment (Guo et al., 2007). The ecological risk of the Daliaohe River estuary is relatively high. Therefore, comparative study between the Daliaohe and Liaohe river estuaries can provide extensive research opportunity regarding the fate of ARGs in the estuary environment under the effects of different anthropogenic activities.
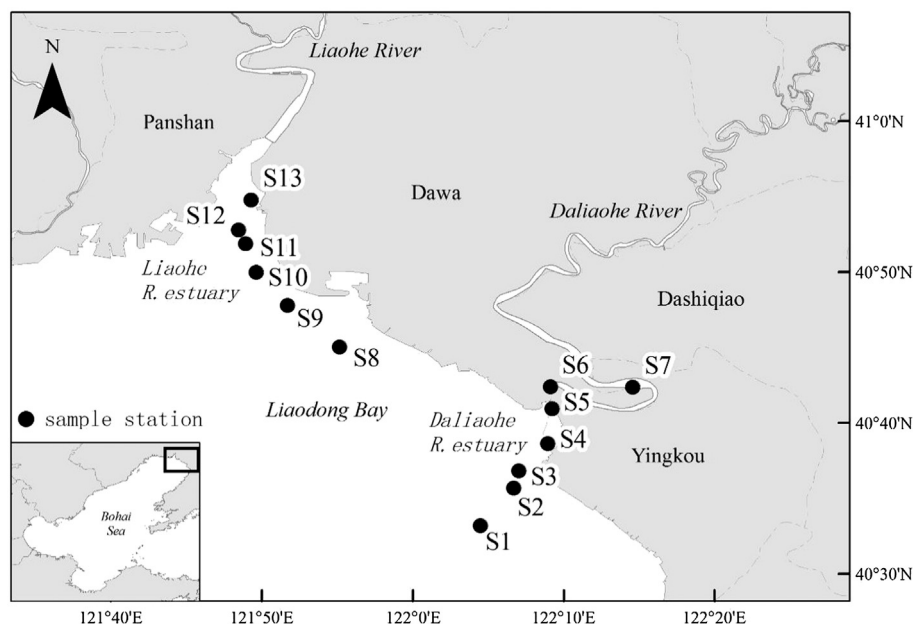


**Fig. 1.** Location of the sampling sites.