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Persistence and risk of antibiotic residues and antibiotic resistance genes in major mariculture sites in Southeast China

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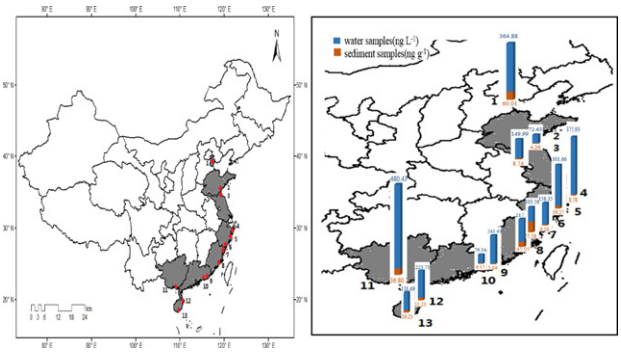
HIGHLIGHTS

- 11 antibiotics were detected in water and sediments from mariculture sites in China.
- Water samples were dominated by sulfadiazine.
- Sediment samples were dominated by doxycyclinehyclate.
- Antibiotic resistance genes were dominated by *sul*2 in both water and sediments.
- Linear relationships between log K_{oc} and log K_{ow}, and between log K_{oc} and log MW.

GRAPHICAL ABSTRACT



Total concentrations of the target antibiotics in water and sediments from mariculture sites in SE China.

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ABSTRACT

Antibiotics are widely used in mariculture industry, and this study attempts to determine the extent of water and sediment pollution by antibiotic residues in 13 major mariculture sites in China. Through chemical and molecular biology analysis, the results showed that the total concentrations of sulfonamides and tetracyclines were in the range 62.0–373.8 ng L^{-1} and 0.2–259.1 ng L^{-1} respectively in water samples, and in the range 0.19–1.59 ng g^{-1} dry weight and 3.45–74.84 ng g^{-1} dry weight respectively, in sediments samples. The occurrence of antibiotic resistance genes (ARGs) was detected in all sites. Compared with the tetracyclines resistance genes, the absolute copy number and relative abundance of the sulfonamides resistance genes were 4.3 times and 2.3 times higher in water and sediment from the mariculture sites, with the dominant resistance genes being *sul*2. The abundance of *sul*3 in the water phase was significantly correlated with the concentrations of sulfamerazine, while the abundance of *sul*2 in the sediment phase was significantly with sulfadiazine concentrations. The abundance of *tet*M in the sediment phase was significantly correlated with the concentrations of oxytetracycline. The findings demonstrate the persistence of antibiotic residues and ARGs in major mariculture sites in Southeast China.

1. Introduction

As significant amounts of antibiotics are used and subsequently released into the aquatic and terrestrial environments (Ahmed et al., 2015), the occurrence and potential environmental impact of antibiotic

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residues are a research focus today worldwide. Among different sources of input, antibiotic residues are mainly discharged from municipal sewage treatment plants and animal husbandry (Heuer et al., 2011; Zhou et al., 2009; García-Galán et al., 2011; Grover et al., 2011). Due to their different physicochemical properties, antibiotics may show different degradation rates in the environment, with some compounds such as sulfonamides being highly persistent (Li et al., 2012; Zhou and Broodbank, 2014), and therefore their contamination levels in the environment have been increasing.

Along with the rapid development of fish farming, the application of antibiotics in the aquaculture industry is becoming more and more extensive for the effective control of the aquatic disease and ensuring profitable aquaculture (Xue et al., 2013). Due to insufficient scientific knowledge from fish farmers and the lack of stringent control measures, excessive antibiotic use in aquaculture industry is widespread hence acting as a pollution source in the marine environment (Arias and Murray, 2009). This is a particularly serious problem in China with extensive fish farming industry, which supplies fish proteins for its massive population and is part of national strategy for food security (Mo et al., 2016).

Antibiotics such as sulfonamides and tetracyclines have been used widely to treat bacterial and protozoan infections in aquaculture (Gao et al., 2012a), resulting in the occurrence of different residues in aquaculture (Gao et al., 2012a; Cheng et al., 2013). In addition to antibiotic pollution in aquaculture environments and neighbouring coastal waters, antibiotic resistance gene (ARGs) may be induced by plasmid horizontal transfer mechanism (Sengelov et al., 2003). The migration, and transformation of ARGs in the aquatic environment is potentially more harmful than the antibiotic residues in the environment (Perreten and Boerlin, 2003; Arias and Murray, 2009; Ji et al., 2012). More attention should be given to sulfonamide antibiotics because of their widespread use, high excretion rate, high solubility and persistence in the environment (García-Galán et al., 2011); for example it is reported that sulfonamide-resistant bacteria can remain stable in the aquatic environment for 5–10 years (Enne et al., 2001; Martinez et al., 2009). Gao et al. (2012b) reported that significant correlations were observed between tetracycline and sulfamethoxazole resistant bacteria and corresponding antibiotic concentrations in wastewater; however, no significant correlations were observed between total *tet* genes (*tet*O and *tet*W) and total concentration of tetracyclines. It is therefore of great importance to address the occurrence of antibiotic residues and ARGs in aquaculture (Xiong et al., 2015; Di Cesare et al., 2013), and their potential relationship in environmental samples. In addition, many studies reported significant impacts that antibiotics may cause on aquatic organisms such as their survival, growth and body weight (Backhaus and Grimme, 1999; Wollenberger et al., 2000; Ji et al., 2012).

This study aimed to quantify antibiotic residues and ARGs in major mariculture areas in China's southeast coast, well known for their large-scale fish farming activities. Specifically the levels and distribution characteristics of widely used antibiotics in mariculture sites were determined; the type and abundance of ARGs was established; the correlation between the level of antibiotic residues and ARGs was assessed; and the overall risks from antibiotic residues in mariculture in Southeast China were evaluated.

2. Materials and methods

2.1. Sampling sites

Sampling was conducted in March 2013 and January 2014, from 13 major mariculture locations in China (Fig. 1), including Tianjin (TJ, site 1), Qingdao (QD, site 2) in Shandong Province, Lianyungang (LYG, site 3) in Jiangsu Province, Zhoushan (ZS, site 4), Ninghai (NH, site 5) and Wenzhou (WZ, site 6) in Zhejiang Province, Ningde (ND, site 7) and Quanzhou (QZ, site 8) in Fujian Province, Shantou (ST, site 9) and Shenzhen (SZ, site 10) in Guangdong Province, Fangchenggang (FCG, site 11) in Guangxi Province, Haikou (HK, site 12) and Sanya (SY, site 13) in Hainan Province. The reference sites were selected from the areas near the fish farming sites with no farming operations wherever possible. A summary of the sampling and reference sites is shown in Supplementary material Table A1. In order to ensure the comparability of the data, all the chosen sites had sea bass (*Lateolabrax japonicus*) as the main fish being farmed.

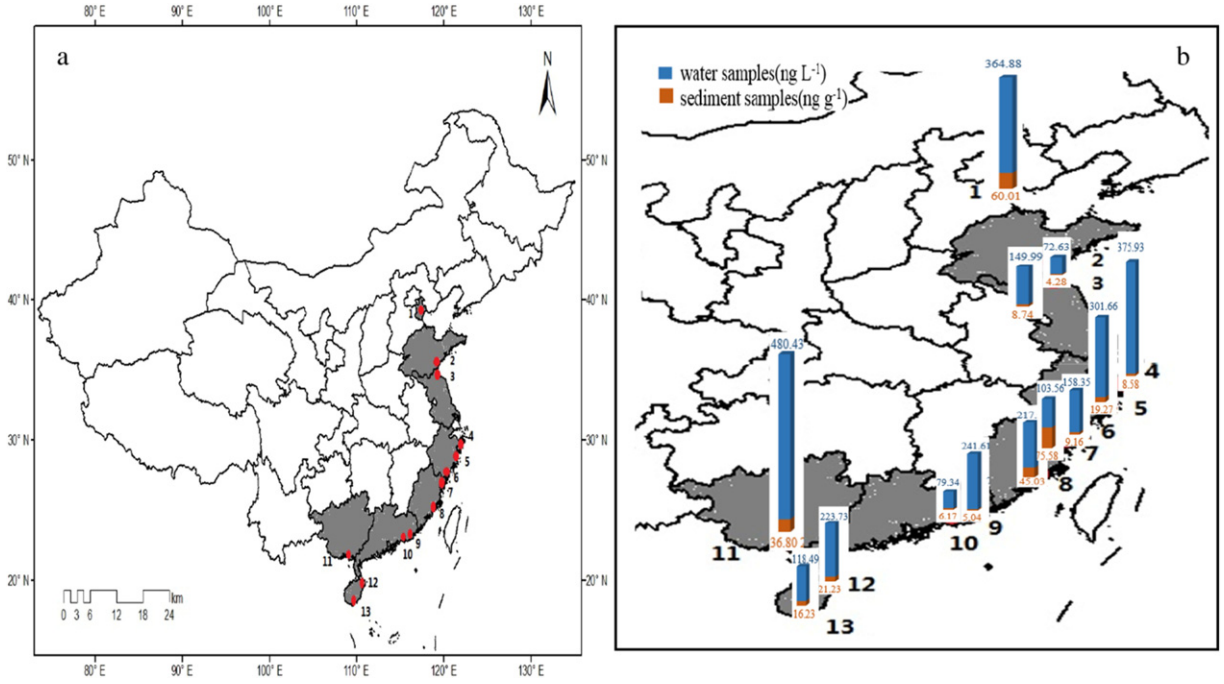

Fig. 1. (a): The sampling locations of the major mariculture sites in China. There are site 1 (Tianjin), site 2 (Qingdao), site 3 (Lianyungang), site 4 (Zhoushan), site 5 (Ninghai), site 6 (Wenzhou), site 7 (Ningde), site 8 (Quanzhou), site 9 (Shantou), site 10 (Shenzhen), site 11 (Fangchenggang), site 12 (Haikou) and site 13 (Sanya). (b): Spatial distribution of total antibiotic concentrations in water and sediment samples.

2.2. Chemical standards

The antibiotics standards sulfonamides (SAs) including sulfadiazine (SD), sulfapyridine (SP), sulfamethoxazole (SMX), sulfathiazole (ST), sulfaquinoxaline (SQ), sulfamethazine (SMT) and sulfamerazine (SM), and tetracyclines (TCs) including tetracycline (TC), oxytetracycline (OTC), doxycycline (DXC) and chlortetracycline (CTC) were purchased from Dr. Ehrenstorfer (GmbH, Germany). The internal standards sulfamethoxazole-d_4 and demeclocycline for quantifying sulfonamides and tetracylines, respectively, were obtained from Dr. Ehrenstorfer (GmbH, Germany). From these individual standards, a 10 mg L^{-1} mixture of working standards containing all compounds was prepared by diluting the stock solution with methanol. All standard solutions were stored at −20 °C. All solvents used were of HPLC grade from Tedia Ltd., USA.

2.3. Sample collection and preparation for antibiotic analysis

For antibiotic residue experiments, all sampling equipment was thoroughly cleaned with acetone, then deionized water and Milli-Q water prior to fieldwork. Water samples (6 L) were collected in triplicate fish cages using glass bottles, while sediment samples (100 g) in triplicate were collected from the first layer of sediment using Van Veen grab, wrapped in aluminium foil before being stored in plastic bags in a freezer. The water samples were immediately filtered through glassfibre filters of 1-μm pore size (Pall, USA). The filtrate was acidified to pH 4 by adding drops of 0.1 M citrate buffer, then supplemented with 0.2 g L^{-1} Na_2EDTA as a chelating agent, and finally spiked with 50 ng of internal standards. The filtered water samples were concentrated by solid-phase extraction (SPE) using Oasis HLB SPE cartridge (200 mg, 6 mL) obtained from Waters, following developed methods (Zhou et al., 2012; Chen and Zhou, 2014). Briefly the SPE cartridges were conditioned with 10 mL each of methanol and Milli-Q water, before the water samples were percolated at a flow rate < 10 mL min^{-1}. After extraction, the antibiotics were eluted twice from the SPE cartridges by methanol (5 mL). The extracts were evaporated under N_2 to 0.5 mL before analysis.

The sediment samples were freeze dried for 72 h before extraction by ultrasonication twice (Chen and Zhou, 2014). Briefly dry sediment (3 g) was mixed with 9 mL of a buffer solution (trisodium phosphate dodecahydrate, sodium citrate), sonicated for 30 min, and centrifuged at 2500 rpm for 5 min. All the supernatant was combined, blown down under a gentle N_2 stream to 0.5 mL before analysis. In addition, sediments were analyzed for particulate organic carbon content by treating with sulfurous acid (5%) twice. The sediment samples were then analyzed for elemental composition after high-temperature catalytic combustion (Zhou et al., 1999).

2.4. Chemical analysis of antibiotics

The target antibiotics were analyzed by a Waters Acquity ultra high performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS) system in multiple reaction monitoring (MRM) mode (Chen and Zhou, 2014). The target compounds were separated on a Waters HSS T3 column (100 mm × 2.1 mm, i.d. 1.8 mm) after 4 μL of sample injection. The mobile phase of eluent A (0.1% formic acid in ultra-pure water) and eluent B (acetonitrile containing 0.1% formic acid) was used, with a gradient program of 85% A (0 min), 83% A (3 min), 70% A (7 min), 35% A (1 min), 100% B (10 min), and finally 85% A (12 min). The column temperature was maintained at 40 °C and the flow rate was 0.4 mL min^{-1}. Mass spectrometric analysis was conducted on a Waters triple quadrupole tandem mass spectrometer with a Z-spray electrospray interface (Waters Corp., Manchester, UK) in positive ionization with optimized capillary voltage (3.0 kV), cone voltage (6–100 V), source temperature (150 °C), desolvation gas temperature (500 °C), desolvation gas flow (800 L h^{-1} nitrogen), cone gas flow (150 L h^{-1} nitrogen) and argon collision gas flow (0.17 mL min^{-1}). The limits of quantification (LOQs) measured as the concentrations corresponding to a signal to noise ratio of 10 were estimated to be between 0.03 and 1.20 ng L^{-1} for water samples, and between 0.01 and 0.40 ng g^{-1} dry weight for sediment samples, as summarized in Supplementary material Table A2 (Chen and Zhou, 2014). In addition, the internal standard demeclocycline was not detected in any batch of the water and sediment samples, confirming its suitability for the role.

2.5. DNA extraction and PCR assays for detection of ARGs

All samples were collected using containers which had been sterilized by autoclaving. Water samples (1.5 L) were collected in triplicate using plastic bottles, while sediment samples (100 g) in triplicate were collected and wrapped in aluminium foil before being stored in plastic bags. All the samples were immediately placed on ice before pretreatment. Water samples (0.5 L) were filtered through a 0.45 μm filter under vacuum, and the filters placed in extraction tubes in the Ultraclean Water DNA Kit (MoBio Laboratories, Inc.). DNA was extracted according to manufacturer's protocol. DNA from 1 g of lyophilized sediment samples was extracted with the Power-Soil DNA Isolation Kit (MoBio Laboratories, Inc.) according to manufacturer's protocol. The extracted DNA quality was checked by running 1% agarose gel and the concentration was measured using an ultraviolet-visible spectrophotometer (Merinton SMA4000, Beijing, China). About 100 ng DNA from each sample was added into a 25 μL PCR reaction system. PCR products of sulfonamide resistance genes (*sul*1, *sul*2, *sul*3), tetracycline resistant genes (*tet*A, *tet*B, *tet*M, *tet*O, *tet*Q, *tet*W) and reference gene (16S rDNA) were cloned into TA vector and then transfected into component *E. coli* cells (ThermoFisher Scientific, Carlsbad, CA, USA). The plasmids containing target and reference gene fragments were then purified and confirmed by sequencing. All plasmids were further gradually diluted and used as Quantitative real-time PCR standards and positive controls. Negative control (DNA being replaced by Milli-Q water) was also included in PCR analysis. The PCR primers for each gene and the PCR program were chosen according to a study by Gao et al. (2012a). All qPCR assays were performed in 20 μL reaction mixtures (SYBER Green real time master mix, Takara, Dalian, China) using an ABI-7500 instrument (Applied Biosystems, Foster City, CA, USA).

2.6. Statistical analysis

Two tailed Student *t*-test was used to compare the means of two independent samples. Least-square linear regression analysis was used to assess the correlation between the antibiotic concentration and the log-odds of resistance. The Spearman coefficient of determination (r^2) and its corresponding *P* value was calculated. *P* values <0.05 were considered significantly different.

3. Results and discussion

3.1. Antibiotic residues in water

The detection rate of SAs was 100% while the detection rate of TCs was only 69.2% in the water samples from mariculture sites (Table 1). The total concentrations of all target antibiotics in mariculture waters varied between 72.6 ng L^{-1} at site 2 (Qingdao) and 480.4 ng L^{-1} at site 11 (Fangchenggang), indicating widespread antibiotic pollution in these mariculture sites in China (Fig. 1). In addition to site 11, the other most highly contaminated locations are site 4 (375.9 ng L^{-1}), site 1 (364.9 ng L^{-1}) and site 5 (301.7 ng L^{-1}) all showing total antibiotic concentrations of over 300 ng L^{-1}. Such high contamination levels are a reflection of extensive and intensive mariculture activities in Fangchenggang, Zhoushan, Tianjin and Ninghai as a major local industry. The dominant classes of contaminants were the SAs, accounting

Table 1
Summary of antibiotic concentrations in (a) water samples (ng L^{-1}) and (b) sediment samples (ng g^{-1}) in mariculture sites in China.

	SAs (n = 13)							TCs (n = 13)			
	SD	SP	SMX	ST	SM	SMT	SQ	TC	OTC	DXC	CTC
(a) Water samples (ng L^{-1})											
Freq (%)	100	100	100	100	100	100	100	54	54	77	92
Max.	291.2	47.9	69.5	121.5	2.3	63.9	19.0	54.7	158.6	28.7	27.4
Min.	17.2	3.7	7.4	n.d.	<LOQ	7.3	3.69	n.d.	n.d.	n.d.	n.d.
Mean	82.9	16.0	22.0	19.1	0.2	25.5	9.1	9.3	22.6	9.7	5.7
RSD (%)	77.9	12.9	16.8	36.2	0.6	16.7	4.6	16.8	45.0	11.4	8.8
(b) Sediment samples (ng g^{-1})											
Freq (%)	46.1	84.6	76.9	23.1	100	100	100	100	100	100	100
Max.	0.40	0.22	0.35	0.08	0.09	0.80	0.13	25.33	39.50	40.57	0.87
Min.	n.d.	n.d.	n.d.	n.d.	0.02	<LOQ	0.01	0.87	0.43	0.25	0.12
Mean	0.12	0.09	0.13	0.03	0.05	0.26	0.04	6.45	7.40	9.34	0.42
RSD (%)	0.14	0.07	0.10	0.02	0.02	0.23	0.03	7.30	11.44	14.08	0.25

for 41–99% of total concentrations among the monitored compounds in the water phase. The average concentrations of SAs and TCs were 25.0 ng L^{-1} and 11.8 ng L^{-1} in the water samples.

In the group of SAs, all the compounds SD, SP, SMX ST, SM, SMT and SQ showed 100% detection. The highest concentrations among the seven compounds were SD and ST, reaching 291.2 ng L^{-1} and 121.5 ng L^{-1}, respectively (Table 1). Compared with previous studies of SAs in coastal aquaculture areas (Table 2), the concentrations of contaminants in this study are higher than mariculture sites in the Beibu Gulf, China with maximum concentrations of 3.41, 10.4 and 0.76 ng L^{-1} for SD, SMX and ST (Zheng et al., 2012), but similar to sites like Laizhou Bay, Guangdong and Bohai Bay, China (Zou et al., 2011; Zhang et al., 2012; Xiong et al., 2015) and Mediterranean, Spain (Moreno-González et al., 2015). However, even higher concentrations were reported in mariculture sites in Northern Vietnam, with the maximum concentrations of 914 ng L^{-1} for SMX (Hoa et al., 2011). Such different concentrations can be caused by the different amount of pharmaceuticals being used and the local environmental conditions such as temperature and hydrodynamics which will affect the persistence and degradation of such chemicals.

In the group of TCs, the four antibiotics were frequently detected (54–92% frequency). As shown in Fig. 2, the concentration of TCs in the 13 mariculture sites was highly variable, which ranged from 0.15 ng L^{-1} (Qingdao) to 259.1 ng L^{-1} (Fangchenggang). The concentration of CTC varied between n.d. and 27.4 ng L^{-1} in the mariculture sites, which is similar to the result (29.1–48.6 ng L^{-1}) reported in the mariculture sites in Guangdong, China (Xiong et al., 2015). The maximum concentration of TC in water samples (54.7 ng L^{-1}) was higher than that in Haihe River at ≤2.70 ng L^{-1} (Luo et al., 2010) and Yellow River at ≤18.0 ng L^{-1} (Zhou et al., 2011). As shown in Fig. 2, tetracyclines had a relatively low concentration in some of the 13 mariculture sites, including Qingdao (0.15 ng L^{-1}), Lianyungang (1.19 ng L^{-1}), Zhoushan (2.04 ng L^{-1}), Ninghai (3.09 ng L^{-1}), Wenzhou (4.35 ng L^{-1}), Ningde (5.10 ng L^{-1}), Shantou (0.91 ng L^{-1}) and Shenzhen (0.65 ng L^{-1}). These eight mariculture sites therefore had much better water quality than the other five sites in terms of tetracycline antibiotic residues.

In terms of the contamination profiles in reference sites, the target compounds were less frequently detected. For SAs and TCs, their detection rates were 78% and 43%, respectively. In addition, the

Table 2
A comparison of antibiotic concentrations in (a) water samples (ng L^{-1}) and (b) sediment samples (ng g^{-1}) from different mariculture sites.

	This study	Guangdong, China	Bohai Bay, China	Beibu Gulf, China	Laizhou Bay, China	Western Thailand	Northern Vietnam	Brittany, France	Mediterranean, Spain
(a) Water samples (ng L^{-1})									
SD	17.2–291.2	n.a.	<LOQ	n.d.–3.4	n.d.–0.4	n.a.	n.a.	n.a.	n.a.
SP	3.7–47.9	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
SMX	7.4–69.5	n.a.	<LOQ–140	n.d.–10.4	1.5–82	n.a.	n.d.–914	n.a.	n.d.–94
ST	n.d.–121.5	n.a.	n.a.	n.d.–0.8	n.a.	n.a.	n.d.	n.a.	n.a.
SM	<LOQ–2.3	0.7–3.6	n.a.	n.a.	n.a.	n.a.	n.d.	n.a.	n.a.
SMT	7.3–63.9	n.a.	n.a.	n.a.	n.a.	n.a.	n.d.	n.a.	n.a.
SQ	3.7–19.0	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
TC	n.d.–54.7	n.a.	<LOQ–30	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
OTC	n.d.–158.6	n.a.	<LOQ–270	n.a.	n.a.	n.d.	n.a.	n.d.	n.a.
DXC	n.d.–28.7	n.d.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
CTC	n.d.–27.4	29.1–48.6	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
(b) Sediment samples (ng g^{-1} dry weight)									
SD	n.d.–0.4	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
SP	n.d.–0.2	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
SMX	n.d.–0.4	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.d.
ST	n.d.–0.1	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
SM	0.02–0.1	3.0–5.6	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
SMT	<LOQ–0.8	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
SQ	0.01–0.1	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
TC	0.9–25.3	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
OTC	0.4–39.5	0.5–3.2	n.a.	n.a.	n.a.	6.0–4062	n.a.	<LOQ–1500	n.a.
DXC	0.3–40.6	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
CTC	0.1–0.9	0.5–2.4	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Ref.	This study	Xiong et al., 2015	Zou et al., 2011	Zheng et al., 2012	Zhang et al., 2012	Rico et al., 2014	Hoa et al., 2011	Pouliquen et al., 2009	Moreno-González et al., 2015

n.d. = not detected, n.a. = not analyzed.

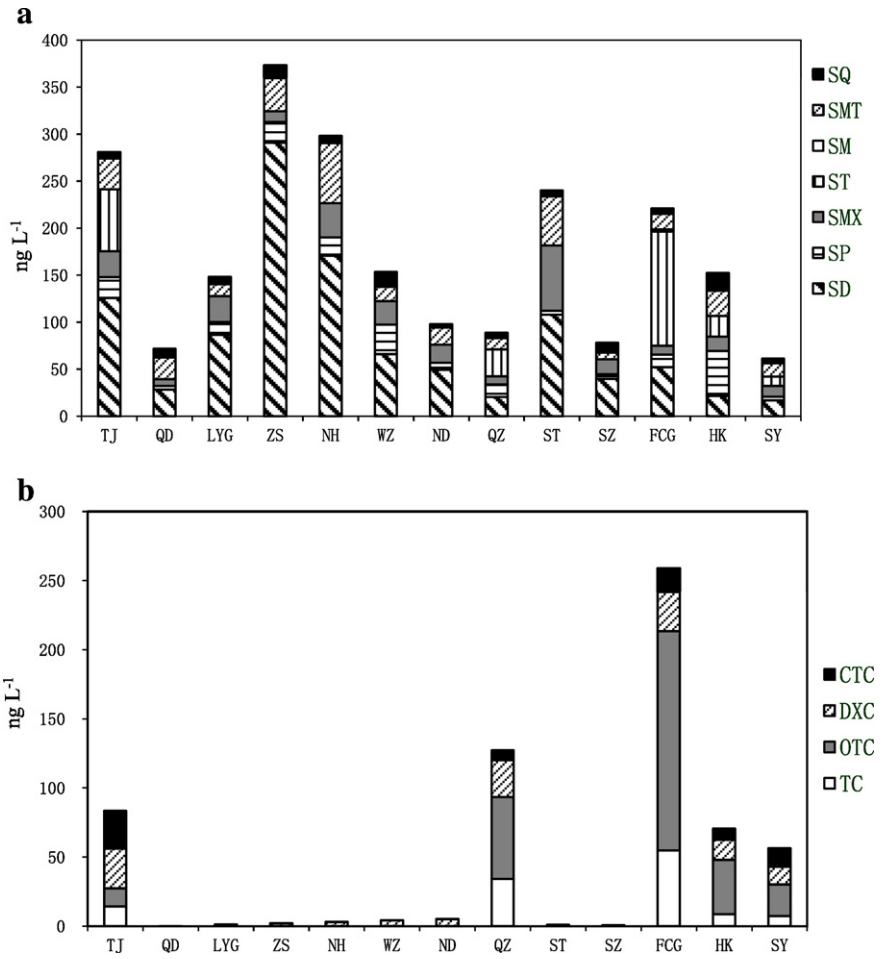

Fig. 2. The concentrations of (a) sulfonamides and (b) tetracyclines in the water phase of mariculture sites in China.

concentrations of the target compounds were lower in reference sites than in sampling sites, as shown in Supplementary material Fig. A1. It is likely that fish farming may have caused an increase in antibiotic concentrations in the surrounding waters.

3.2. Antibiotic residues in sediment

The TCs were detected in 100% of sediment samples, while the SAs showed a detection rate of 23.1% to 100% in sediment phase. The total concentration of all target antibiotics ranged from 4.28 ng g^{-1} (site 2) to 75.58 ng g^{-1} (site 7), all on dry weight basis. In addition to site, 7, site 1 (60.01 ng g^{-1}), site 8 (45.03 ng g^{-1}) and site 11 (36.80 ng g^{-1}) are the most heavily contaminated sites in sediment environment. Different from the water phase, the TCs were the dominant contaminants in the sediments of these mariculture sites (Fig. 3), contributing 68% to 99% to the total antibiotic burden from all sites.

As shown in Table 2, in the group of SAs, the maximum concentrations of individual compounds SD, SP, SMX, ST, SM, SMT and SQ were 0.1–0.8 ng g^{-1}, which are similar to those reported in many other studies. The relatively low concentrations of SAs in sediments coincided with those by Zhang et al. (2013) and Chen and Zhou (2014), which suggested that sulfonamides had a weak sediment adsorption property. In comparison, Na et al. (2013) reported higher concentrations of antibiotics in marine sediments, e.g. SD at 82.48 ng g^{-1}, ST at 72.87 ng g^{-1}, SM at 91.90 ng g^{-1} and SMX at 90.33 ng g^{-1}, than those observed in this study. Although ST was prohibited in fish farming in China, it was detected in all water samples and 23.1% of sediment samples, suggesting a clear violation of governmental guidelines in fish farming practice.

The concentrations of TCs varied from 0.1 to 40.6 ng g^{-1}, which are similar to those detected in the Yangtze Estuary and its coastal areas (Shi et al., 2014). However, Rico et al. (2014) observed up to 4062 ng g^{-1} of OTC in the sediments collected from tilapia cage farming in Thailand. Pouliquen et al. (2009) also reported up to 1500 ng g^{-1} of OTC in sediments from Brittany, France. The detection rate of TCs was 100% for all compounds, suggesting their widespread occurrence. As shown in Table 1, the mean concentrations of TC, OTC and DXC were 6.45, 7.40 and 9.34 ng g^{-1}, which were one order of magnitude higher than those of CTC (0.42 ng g^{-1}). In comparison, such levels are similar to those detected in the sediments of the Yangtze River Estuary, China (Shi et al., 2014).

In comparison to the sampling sites, the target compounds were less frequently detected in the reference sites, with an average of 81% and 66% detection for TCs and SAs, respectively. Furthermore, the concentrations of the target antibiotics were lower in reference sites than in sampling sites (Supplementary material Fig. A1).

3.3. Sediment-water distribution of antibiotics

Sediment-water interaction plays a key role in regulating the distribution, persistence and long-term fate of contaminants in the aquatic environment (Zhou et al., 1999). From the antibiotic concentrations determined in both sediment and water samples, the partition coefficient between sediment and water (K_p) can be estimated as follows:

$$K_p = C_s / C_w \quad (1)$$

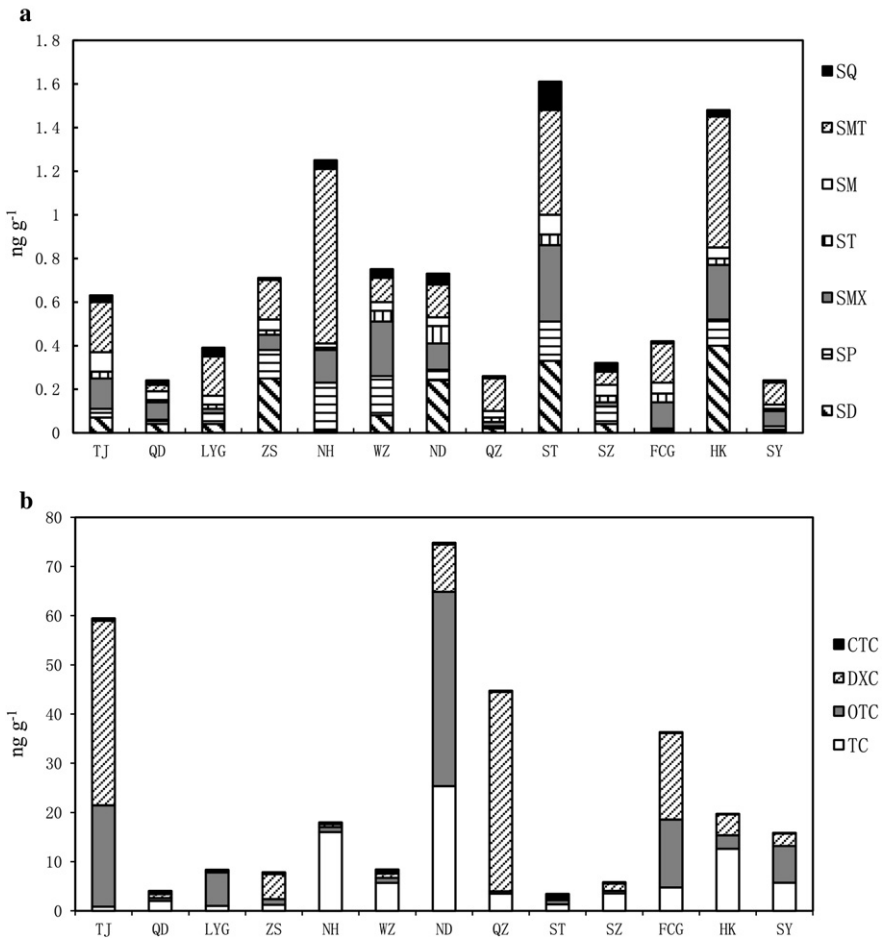

Fig. 3. The concentrations of (a) sulfonamides and (b) tetracyclines in the sediment phase of mariculture sites in China.

where C_s and C_w are the antibiotic concentrations in sedimentary and water phases, respectively.

Such values provide estimates of K_p as true partition equilibrium may not have been reached under field conditions (Moreno-González et al., 2015). Previous studies showed that the distribution of many organic contaminants between sediment and water phases is significantly affected by the concentration of inorganic cations (Schaffer et al., 2012), temperature (Smith and McLachlan, 2006), particles (Aminot et al., 2015) and labile/refractory organic matter (Belles et al., 2016). Due to the widely reported important role by sediment organic carbon (e.g. Chen and Zhou), the organic carbon normalized partition coefficient (K_{oc}) was calculated by Eq. (2):

$$K_{oc} = K_p / f_{oc} \quad (2)$$

where f_{oc} is fraction organic carbon content in sediments.

The K_p values were highly variable, ranging from 3.6 to 2934 L kg^{-1} for ST, 23.3 to 1774 L kg^{-1} for SM, 11.3 to 123,509 L kg^{-1} for CTC. Such a variability is likely to be due to several factors including differences in the local hydrodynamic regimes, suspended sediment concentrations (Zhou and Broodbank, 2014), and the types of sediments with varying particle size and organic matter composition (Zhou et al., 1999). To account for the difference in sediment organic carbon content, the K_{oc} values were calculated using Eq. (2). The calculations show that K_{oc} values varied from 14.1 to 5394 L kg-oc^{-1} for SP, 61 to 40,926 L kg-oc^{-1} for ST, and 36.8 to 2521 L kg-oc^{-1} for SQ. The findings suggest that although sediment organic carbon content is an important parameter in sediment/water interactions of antibiotics, there are additional factors such as complexity of sediment (Moreno-González et al., 2015) and chemical speciation which also affect or even control such interactions and need further research. For example, many antibiotic compounds are amphoteric molecules having ionizable functional groups, therefore their interaction with sediment will be highly dependent on the form (i.e. neutral or charged) in which the antibiotics are present and the surface charge of sediment sorbents (Zhao et al., 2016). To identify such additional factors, the log K_{oc} values were plotted against log K_{ow} values of antibiotics (Fig. 4a). It was observed that there is a linear correlation between log K_{oc} and log K_{ow} for most antibiotics except for two compounds (TC and OTC). This suggested that once the variability of sediment organic carbon content was excluded, the interaction of antibiotics between sediments and water was regulated, to some extent, by the hydrophobicity of antibiotics. In addition, a positive relationship between log K_{oc} and log MW was also observed (Fig. 4b), suggesting that the molecular size of antibiotics also influenced the residue content in sedimentary phases, meaning large and heavy molecules are more likely to be attracted to sediment matrix, consistent with the results reported by Chen and Zhou (2014) in Huangpu River, China. The results also support other studies showing the attraction of antibiotics and other pharmaceuticals to aquatic sediments and colloids which act as a sink for such contaminants (Maskaoui and Zhou, 2010; Zhou and Broodbank, 2014).

3.4. ARGs in water and sediment

ARGs were detected in all sites in this study. The concentrations of each gene (normalized to 16S rDNA) ranged from 9.4×10^{-9} to 6.6×10^{-2} in water samples, and from 3.9×10^{-9} to 7.5×10^{-2} in sediment samples. Compared to tetracycline resistance genes, *sul*2 was the

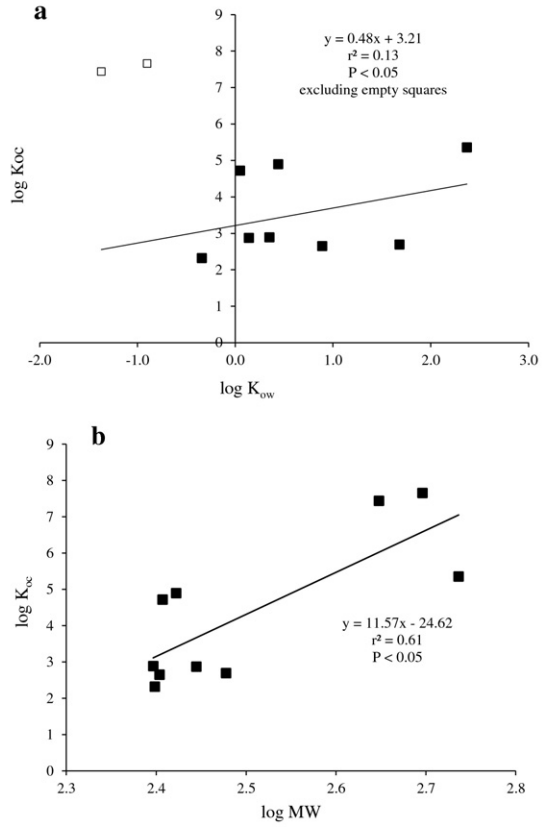

Fig. 4. The relationship between (a) log K_{oc} and log K_{ow} and (b) log K_{oc} and log MW for sulfonamides and tetracyclines in mariculture sites in China.

most abundant in the water and sediment phases among the 9 kinds of target resistant genes in the mariculture sites, with relative expression amount (to internal reference gene 16S rDNA) ranging from 0.029 to 0.075 (Figs. 5a and 6a). As shown in Figs. 5b and 6b, *tet*B was not detected in either water samples or sediment samples, which may be related to the low level of *tet*B in the environment (Auerbach et al., 2007). Some tetracycline resistance genes (*tet*W, *tet*Q, *tet*O, *tet*A, *tet*M) were detected, although less frequently than *sul*1 and *sul*2, the results are similar to those reported in other studies (Zhang et al., 2009; Luo et al., 2010; Xiong et al., 2015).

There was no statistical difference between the relative abundance of sulphonamides resistance genes (*sul*1, *sul*2) in sediments (5.9×10^{-2}) and that in the water samples (8.05×10^{-2}), indicating that the water and sediment environments were polluted by sulfonamide resistance genes to a similar extent. The results are different from those in the Haihe River (Luo et al., 2010), in which ARG (*sul*1, *sul*2) concentrations were approximately 120–2000 times higher in sediments than those in water. The ARGs were also detected in the reference sites, but their abundance was less than in sampling sites, particularly in the sediment phase (Supplementary material Fig. A1).

The relative expression quantity of the resistance gene in the sediment of the mariculture sites declined as: *sul*2 > *sul*1 > *sul*3, *tet*W > *tet*M > *tet*Q > *tet*O > *tet*A. Overall, sulfonamides resistance genes were more abundant than tetracycline resistance genes, reflecting a high usage of sulfonamides resulting in high induction of *sul* genes (Gao et al., 2012a). Such results are consistent with those of Xiong et al. (2015) who reported the relative abundances of *sul* genes in the range 3.4×10^{-4} to 1.1×10^{-2}. The different levels of SAs and TCs resistance genes among various locations would reflect differences in antibiotic usage patterns, residues discharge strengths, water and sediment environment conditions, resulting in different bacterial community evolving in response to prolonged exposure to antibiotic residues. These topics should be further investigated in future research.

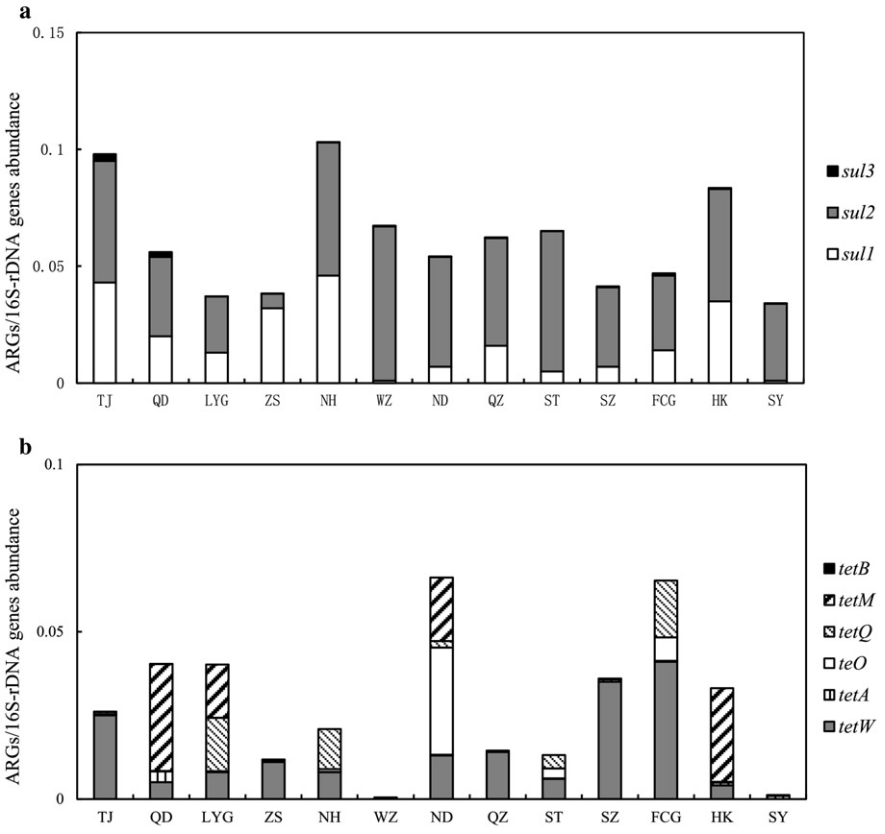

Fig. 5. The abundance of (a) sulfonamides resistance genes and (b) tetracyclines resistance genes in mariculture waters in China.

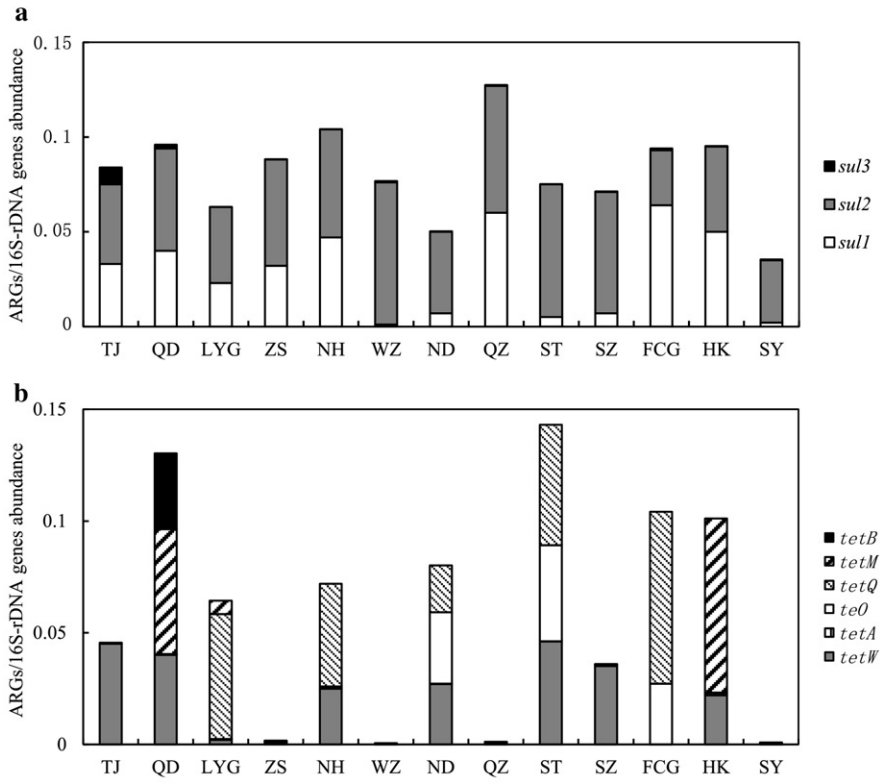

Fig. 6. The abundance of (a) sulfonamides resistance genes and (b) tetracyclines resistance genes in mariculture sediments in China.

3.5. Relationship between antibiotic contamination and ARGs

The use of antibiotics in fish farming is one of the important reasons leading to the increase of drug resistance and hence ARGs in the marine environment (Di Cesare et al., 2013). As shown in Table 3, the relationships between the abundance of *sul*1, *tet*A, *tet*O, *tet*Q, *tet*B and the concentrations of individual antibiotic compound were statistically insignificant ($P > 0.05$). However, *sul*2 abundance in sediments was significantly positively correlated with the concentration of sulfadiazine, and the abundance of *sul*3 in water was significantly positively correlated with the concentrations of sulfamerazine, with $P < 0.05$. Among the TCs resistance genes, *tet*M was the only one significantly related to the concentration of the oxytetracycline in sediment samples ($P < 0.05$).

Table 3
Analysis of the correlation between antibiotic concentrations and ARGs.

	Sample	*sul*1	*sul*2	*sul*3	*tet*W	*tet*A	*tet*O	*tet*Q	*tet*M	*tet*B
SD	Water	−0.307	0.325	0.394	0.140	0.065	0.277	−0.229	0.043	0.035
	Sediment	−0.245	**0.815**	−0.346	−0.088	0.193	−0.115	−0.234	−0.070	−0.345
SP	Water	0.065	0.067	−0.723	0.063	−0.621	−0.164	−0.453	−0.060	−0.316
	Sediment	−0.234	0.578	−0.266	0.161	−0.211	0.075	0.112	−0.053	−0.270
SMX	Water	−0.332	0.360	−0.069	0.085	0.252	−0.297	−0.285	−0.214	−0.266
	Sediment	−0.192	0.301	0.015	0.343	−0.302	−0.302	0.181	0.201	−0.141
ST	Water	0.332	0.360	−0.069	**0.858**	0.252	−0.033	−0.285	−0.367	−0.297
	Sediment	−0.383	0.088	−0.052	0.165	−0.340	−0.340	0.179	−0.209	−0.309
SM	Water	−0.450	−0.332	**0.699**	0.127	0.259	0.282	0.093	0.001	0.136
	Sediment	−0.121	0.105	0.557	0.599	−0.252	−0.252	0.108	−0.028	−0.096
SMT	Water	−0.219	0.367	−0.354	**0.744**	−0.850	0.689	−0.250	−0.230	−0.093
	Sediment	0.290	0.077	−0.117	0.244	−0.026	−0.026	0.336	0.207	−0.286
SQ	Water	0.095	0.069	0.074	0.046	0.053	0.445	−0.076	0.302	0.095
	Sediment	−0.478	0.422	−0.116	0.561	−0.334	−0.334	0.365	−0.129	−0.145
TC	Water	−0.207	0.325	0.309	0.284	−0.372	0.113	−0.065	0.570	−0.290
	Sediment	−0.045	−0.166	−0.272	0.064	−0.085	0.259	0.053	0.091	−0.181
OTC	Water	0.164	−0.310	0.289	0.177	0.009	0.043	−0.016	−0.021	0.029
	Sediment	−0.148	−0.527	0.314	0.143	−0.380	0.435	0.123	**0.923**	−0.180
DXC	Water	0.270	0.472	−0.115	0.197	−0.136	0.045	−0.712	0.374	−0.210
	Sediment	0.483	−0.107	0.428	−0.034	0.128	−0.061	−0.158	−0.217	−0.183
CTC	Water	0.050	0.013	0.057	−0.150	−0.216	0.190	0.223	0.001	−0.449
	Sediment	−0.410	0.640	0.126	0.379	−0.620	0.292	0.054	−0.143	0.287
∑SAs	Water	−0.437	0.728	0.099	**0.764**	0.074	−0.563	−0.112	0.028	0.219
	Sediment	−0.029	0.268	−0.135	0.368	−0.110	0.388	0.234	0.236	−0.289
∑TCs	Water	0.061	−0.232	0.295	0.242	0.147	0.223	0.024	0.015	−0.431
	Sediment	0.202	−0.371	0.428	0.074	−0.143	0.263	−0.018	−0.210	−0.254

Numbers in bold indicate significant difference with $P < 0.05$.

Smith et al. (2004) reported similar results in the feedlot lagoons, USA, that there was no significant correlation between tetracycline resistance genes and tetracycline antibiotics. From the statistical analysis, Pei et al. (2006) also suggested that there was no significant correlation between ARGs and antibiotic concentrations in Poudre River, Colorado, USA. They attributed such findings to the fact that the kinds of resistance genes that can be quantified are less than the resistance genes actually occurring in nature. However, other researchers (Chee-Sanford et al., 2001; Pei et al., 2007) have observed a good correlation between antibiotic concentrations and ARGs. There is no consistent conclusion about the relationship between the abundance of resistance genes and the concentration of antibiotics, suggesting that the concentration of antibiotics may not be the only factor in the environment regulating the resistant gene expression. Engemann et al. (2008) showed that the degradation of resistance genes under light was significantly higher than that in the dark, which fully verified that the light can accelerate the degradation of ARGs. Chen and Zhang (2013) studied the effect of temperature and anaerobic treatment on ARGs content in milk factory wastewater, and the results showed that both high temperature and anaerobic treatment were beneficial to the degradation of ARGs. In addition, genetic selection was proposed as another mechanism for bacteria developing antibiotic resistance (Mah et al., 2003).

3.6. Risk assessment of antibiotic residues

Mariculture is a major industry in many countries including China, and its sustainable growth is crucially important for local and national economies, human nutrition and water environmental protection. Exceedance of antibiotic residues in fish products and their subsequent consumption by human could adversely affect people's health. Therefore the potential risks from antibiotic exposure should be evaluated. Here the hazard quotient (HQ) values for individual contaminants were calculated as follows (Yan et al., 2013):

$$HQ = MEC/PNEC \quad (3)$$

where MEC is the measured environmental concentration PNEC is the predicted no effect concentration. In practice, PNEC was calculated by dividing the ecotoxicity data by an assessment factor (AF), as shown below:

$$PNEC = NOEC/AF \quad (4)$$

where NOEC represents the no observed effect concentration for the most sensitive species, and AF shows whether the toxicity is acute or chronic. In this work, the value of AF was chosen to be 100 to represent chronic toxicity (Leung et al., 2012).

The extent of risk to the living organisms in water is dependent on the values of HQ, which can be classified into three categories: (1) high risk when the HQ exceeds 1, (2) medium risk when the HQ value is between 0.1 and 1, and (3) low risk when the HQ value is between 0.01 and 0.1 (Hernando et al., 2006). In this study, the majority of HQ values were below 0.01 (Fig. 7) in the mariculture sites, indicating the risk from antibiotic residues was insignificant. For compounds SD, SMX and ST, their HQ values for daphnid were higher than 0.01 but lower than 0.1, suggesting low risk. Similar low to medium risk was reported by Yan et al. (2013) and Chen and Zhou (2014) in the Yangtze Estuary and Huangpu River, China. However, they also identified high risk posed by SP and SMX with HQ > 0.1 (Yan et al., 2013; Chen and Zhou, 2014), demonstrating the pollution from these two antibiotics was potentially significant in causing potential ecotoxicological effects. Although such a risk assessment approach is very informative and quantitative, there are limitations as it does not consider the bioavailability of contaminants and potential mixture effects which can cause more significant adverse impact (Leung et al., 2012).

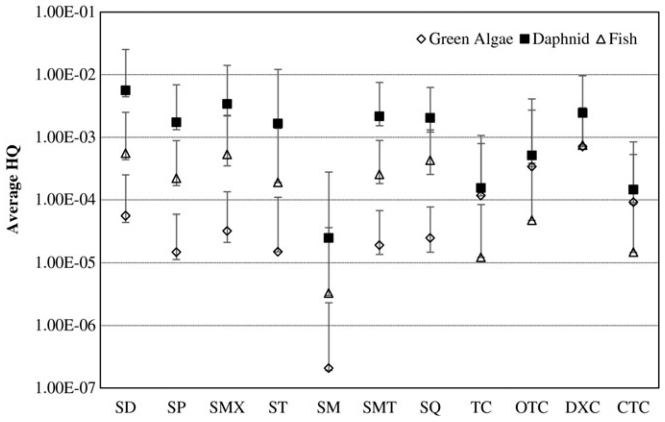

Fig. 7. The risk assessment of antibiotics in the surface water of the 13 mariculture sites in China, showing the average HQ values (symbols) and the maximum and the minimum HQ values (bars).

4. Conclusions

The results demonstrated the occurrence of 11 antibiotic compounds and 9 types of ARGs in the water and sediment phases from 13 major mariculture sites in Southeast China. The main pollutants in the water samples of mariculture sites were sulfonamides, which accounted for 41% to 99% of the total target pollutant burden. The main pollutants in the sediment samples were tetracyclines, which accounted for 68% to 99% of the total monitored pollutant burden. All TCs and SAs resistance genes were detected in mariculture water and sediment samples, with the exception of *tet*B. The detection of sulfonamides and tetracyclines resistance genes showed that *sul* genes were widespread in water and sediment samples, with *sul*2 being the most frequently detected resistance gene in the target mariculture sites in China. The *sul*2 was also the most abundant among the 9 ARGs, with relative abundance ranging from 1.19×10^{-6} to 5.48×10^{-2}. Overall, the average relative expression of resistance genes in water and sediments decreased as: *sul*2 > *sul*1 > *tet*W > *tet*Q > *tet*O > *tet*M > *sul*3 > *tet*A > *tet*B. The correlation analysis between ARGs and the antibiotics showed that only *sul*2 and *sul*3 had significant correlations with the concentrations of sulfonamides (SM and SD), while *tet*M was the only one significantly related to the concentration of OTC. There was no significant correlation between the other antibiotics and the other resistant genes. The implications of the findings are that significant quantities of antibiotic residues and ARGs may eventually be transported into the marine environment through water circulation, sediment suspension and fish movement. Future research should focus on reducing the use of antibiotics in mariculture and controlling the migration of ARGs into the wider environment. The government should develop more stringent standards for regulating the use and monitoring of antibiotics, so that effective control of antibiotic pollution can be realized in such a major industry worldwide.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.scitotenv.2016.12.075.

References

Ahmed, M.B., Zhou, J.L., Ngo, H.H., Guo, W., 2015. Adsorptive removal of antibiotics from water and wastewater: progress and challenges. Sci. Total Environ. 532, 112–126.

Aminot, Y., Litrico, X., Chambolle, M., Arnaud, C., Pardon, P., Budzindki, H., 2015. Development and application of a multi-residue method for the determination of 53 pharmaceuticals in water, sediment, and suspended solids using liquid chromatography-tandem mass spectrometry. Anal. Bioanal. Chem. 407, 8585–8604.

Arias, C.A., Murray, B.E., 2009. Antibiotic-resistant bugs in the 21st century — a clinical super-challenge. New Engl. J. Med. 360, 439–443.

Auerbach, E.A., Seyfried, E.E., McMahon, K.D., 2007. Tetracycline resistance genes in activated sludge wastewater treatment plants. Water Res. 41, 1143–1151.

Backhaus, T., Grimme, L.H., 1999. The toxicity of antibiotic agents to the luminescent bacterium *Vibrio fischeri*. Chemosphere 38, 3291–3301.

Belles, A., Alary, C., Mamindy-Pajany, Y., Abriak, N.E., 2016. Relationship between the water-exchangeable fraction of PAH and the organic matter composition of sediments. Environ. Pollut. 219, 512–518.

Chee-Sanford, J.C., Aminov, R.I., Krapac, I.J., Garrigues-Jeanjea, N., Mackie, R.I., 2001. Occurrence and diversity of tetracycline resistance genes in lagoons and groundwater underlying two swine production facilities. Appl. Environ. Microbiol. 67, 1494–1502.

Chen, H., Zhang, M., 2013. Occurrence and removal of antibiotic resistance genes in municipal wastewater and rural domestic sewage treatment systems in eastern China. Environ. Int. 55, 9–14.

Chen, K., Zhou, J.L., 2014. Occurrence and behavior of antibiotics in water and sediments from the Huangpu River, Shanghai, China. Chemosphere 95, 604–612.

Cheng, W., Chen, H., Su, C., Yan, S., 2013. Abundance and persistence of antibiotic resistance genes in livestock farms: a comprehensive investigation in eastern China. Environ. Int. 61, 1–7.

Di Cesare, A., Luna, G.M., Vignaroli, C., Pasquaroli, S., Tota, S., Paroncini, P., Biavasco, F., 2013. Aquaculture can promote the presence and spread of antibiotic-resistant *Enterococci* in marine sediments. PLoS One 8 (4), e62838.

Engemann, C.A., Keen, P.L., Knapp, C.W., Hall, K.J., Graham, D.W., 2008. Fate of tetracycline resistance genes in aquatic systems: migration from the water column to peripheral biofilms. Environ. Sci. Technol. 42, 5131–5136.

Enne, V.I., Livermore, D.M., Stephens, P., Hall, L., 2001. Persistence of sulphonamide resistance in *Escherichia coli* in the UK despite national prescribing restriction. Lancet 357, 1325–1328.

Gao, P., Mao, D., Luo, Y., Wang, L., Xu, B., Xu, L., 2012a. Occurrence of sulfonamide and tetracycline-resistant bacteria and resistance genes in aquaculture environment. Water Res. 46, 2355–2364.

Gao, P., Mariya, M., Xagoraraki, I., 2012b. Correlation of tetracycline and sulfonamide antibiotics with corresponding resistance genes and resistant bacteria in a conventional municipal wastewater treatment plant. Sci. Total Environ. 421–422, 173–183.

García-Galán, M.J., Díaz-Cruz, M.S., Barceló, D., 2011. Occurrence of sulfonamide residues along the Ebro river basin: removal in wastewater treatment plants and environmental impact assessment. Environ. Int. 37, 462–473.

Grover, D., Zhou, J.L., Frickers, P.E., Readman, J.W., 2011. Improved removal of estrogenic and pharmaceutical compounds in sewage effluent by full scale granular activated carbon: impact on receiving river water. J. Hazard. Mater. 185, 1005–1011.

Hernando, M.D., Mezcua, M., Fernández-Alba, A.R., Barceló, D., 2006. Environmental risk assessment of pharmaceutical residues in wastewater effluents, surface waters and sediments. Talanta 69, 334–342.

Heuer, H., Schmitt, H., Smalla, K., 2011. Antibiotic resistance gene spread due to manure application on agricultural fields. Curr. Opin. Microbiol. 14, 236–243.

Hoa, P.T.P., Managaki, S., Nakada, N., Takada, H., Shimizu, A., Anh, D.H., Viet, P.H., Suzuki, S., 2011. Antibiotic contamination and occurrence of antibiotic-resistant bacteria in aquatic environments of northern Vietnam. Sci. Total Environ. 409, 2894–2901.

Ji, X., Shen, Q., Liu, F., Ma, J., Xu, G., Wang, Y., Wu, M., 2012. Antibiotic resistance gene abundances associated with antibiotics and heavy metals in animal manures and agricultural soils adjacent to feedlots in Shanghai, China. J. Hazard. Mater. 235, 178–185.

Leung, H.W., Minh, T.B., Murphy, M.B., Lam, J.C.W., So, M.K., Martin, M., Lam, P.K.S., Richardson, B.J., 2012. Distribution, fate and risk assessment of antibiotics in sewage treatment plants in Hong Kong, south China. Environ. Int. 42, 1–9.

Li, W., Shi, Y., Gao, L., Liu, J., Cai, Y., 2012. Occurrence of antibiotics in water, sediments, aquatic plants, and animals from Baiyangdian Lake in north China. Chemosphere 89, 1307–1315.

Luo, Y., Mao, D., Rysz, M., Zhou, Q., Zhang, H., Xu, L., Alvarez, P.J.J., 2010. Trends in antibiotic resistance genes occurrence in the Haihe River, China. Environ. Sci. Technol. 44, 7220–7225.

Mah, T.F., Pitts, B., Pellock, B., Walker, G.C., Stewart, P.S., O'Toole, G.A., 2003. A genetic basis for *Pseudomonas aeruginosa* biofilm antibiotic resistance. Nature 426, 306–310.

Martinez, J.L., Fajardo, A., Garmendia, L., Hernandez, A., Linares, J.F., Martínez-Solano, L., Sánchez, M.B., 2009. A global view of antibiotic resistance. FEMS Microbiol. Rev. 33, 44–65.

Maskaoui, K., Zhou, J.L., 2010. Colloids as a sink for certain pharmaceutical in the aquatic environment. Environ. Sci. Pollut. Res. 17, 898–907.

Mo, W.Y., Chen, Z., Leung, H.M., Leung, A.O.W., 2016. Application of veterinary antibiotics in China's aquaculture industry and their potential human health risks. Environ. Sci. Pollut. Res. http://dx.doi.org/10.1007/s11356-015-5607-z (In press).

Moreno-González, R., Rodriguez-Mozaz, S., Gros, M., Barceló, D., León, V.M., 2015. Seasonal distribution of pharmaceuticals in marine water and sediment from a mediterranean coastal lagoon (SE Spain). Environ. Res. 138, 326–344.

Na, G., Fang, X., Cai, Y., Ge, L., Zong, H., Yuan, X., Yao, Z., Zhang, Z., 2013. Occurrence, distribution, and bioaccumulation of antibiotics in coastal environment of Dalian, China. Mar. Pollut. Bull. 69, 233–237.

Pei, R., Cha, J., Carlson, K.H., Amy, P., 2007. Response of antibiotic resistance genes (ARG) to biological treatment in dairy lagoon water. Environ. Sci. Technol. 41, 5108–5113.

Pei, R., Kim, S.C., Carlson, K.H., Pruden, A., 2006. Effect of river landscape on the sediment concentrations of antibiotics and corresponding antibiotic resistance genes (ARG). Water Res. 40, 2427–2435.

Perreten, V., Boerlin, P., 2003. A new sulfonamide resistance gene (sul3) in *Escherichia coli* is widespread in the pig population of Switzerland. Antimicrob. Agents Chemother. 47, 1169–1172.

Pouliquen, H., Delepee, R., Thorin, C., Haury, J., Larphantec-Verdier, M., Morvan, M.-L., Bris, H.L., 2009. Comparison of water, sediment, and plants for the monitoring of antibiotics: a case study on a river dedicated to fish farming. Environ. Toxicol. Chem. 28, 496–592.

Rico, A., Oliveira, R., McDonough, S., Matser, A., Khatikarn, J., Satapornvanit, K., Nogueira, A.J.A., Soares, A.M.V.M., Domingues, I., Van den Brink, P.J., 2014. Use, fate and ecological risks of antibiotics applied in tilapia cage farming in Thailand. Envion. Pollut. 191, 8–16.

Schaffer, M., Börnick, H., Nödler, K., Licha, T., Worch, E., 2012. Role of cation exchange processes on the sorption influenced transport of cationic β-blockers in aquifer sediments. Water Res. 46, 5472–5482.

Sengelov, G., Agerso, Y., Halling-Sorensen, B., Baloda, S.B., Andersen, J.S., Jensen, L.B., 2003. Bacterial antibiotic resistance levels in Danish farmland as a result of treatment with pig manure slurry. Environ. Int. 28, 587–595.

Shi, H., Yang, Y., Liu, M., Yan, C., Yue, H., Zhou, J., 2014. Occurrence and distribution of antibiotics in the surface sediments of the Yangtze Estuary and nearby coastal areas. Mar. Pollut. Bull. 83, 317–323.

Smith, K.E.C., McLachlan, M.S., 2006. Concentrations and partitioning of polychlorinated biphenyls in the surface waters of the southern Baltic Sea—seasonal effects. Environ. Toxicol. Chem. 25, 2569–2575.

Smith, M.S., Yang, R.K., Knapp, C.W., 2004. Quantification of tetracycline resistance genes in feedlot lagoons by real-time PCR. Appl. Environ. Microbiol. 70, 7372–7377.

Wollenberger, L., Halling-Sorenson, B., Kusk, K.O., 2000. Acute and chronic toxicity of veterinary antibiotics to *Daphnia magna*. Chemosphere 40, 723–730.

Xiong, W., Sun, Y., Zhang, T., Ding, X., Li, Y., Wang, M., Zeng, Z., 2015. Antibiotics, antibiotic resistance genes, and bacterial community composition in fresh water aquaculture environment in China. Microb. Ecol. 70, 425–432.

Xue, B., Zhang, R., Wang, Y., Liu, X., Li, J., Zhang, G., 2013. Antibiotic contamination in a typical developing city in south China: occurrence and ecological risks in the Yongjiang River impacted by tributary discharge and anthropogenic activities. Ecotoxicol. Environ. Safe. 92, 229–236.

Yan, C., Yang, Y., Zhou, J., Liu, M., Nie, M., Shi, H., Gu, L., 2013. Antibiotics in the surface water of the Yangtze estuary: occurrence, distribution and risk assessment. Environ. Pollut. 175, 22–29.

Zhang, R., Tang, J., Li, J., Cheng, Z., Chaemfa, C., Liu, D., Zheng, Q., Song, M., Luo, C., Zhang, G., 2013. Occurrence and risks of antibiotics in the coastal aquatic environment of the Yellow Sea, north China. Sci. Total Environ. 450–451, 197–204.

Zhang, R., Zhang, G., Zheng, Q., Tang, J., Chen, Y., Xu, W., Zou, Y., Chen, X., 2012. Occurrence and risks of antibiotics in the Laizhou Bay, China: impacts of river discharge. Ecotoxicol. Environ. Safe. 80, 208–215.

Zhang, X., Zhang, T., Fang, H.H.P., 2009. Antibiotic resistance genes in water environment. Appl. Microbiol. Biotechnol. 82, 397–414.

Zhao, H., Liu, X., Cao, Z., Zhan, Y., Shi, X., Yang, Y., Zhou, J., Xu, J., 2016. Adsorption behavior and mechanism of chloramphenicols, sulfonamides, and non-antibiotic pharmaceuticals on multi-walled carbon nanotubes. J. Hazard. Mater. 310, 235–245.

Zheng, Q., Zhang, R., Wang, Y., Pan, X., Tang, J., Zhang, G., 2012. Occurrence and distribution of antibiotics in the Beibu Gulf, China: impacts of river discharge and aquaculture activities. Mar. Environ. Res. 78, 26–33.

Zhou, J., Broodbank, N., 2014. Sediment-water interactions of pharmaceutical residues in the river environment. Water Res. 48, 61–70.

Zhou, J.L., Fileman, T.W., Evans, S., Donkin, P., Readman, J.W., Mantoura, R.F.C., Rowland, S., 1999. The partition of fluoranthene and pyrene between suspended particles and dissolved phase in the Humber estuary: a study of the controlling factors. Sci. Total Environ. 244, 305–321.

Zhou, J.L., Maskaoui, K., Lufadeju, A., 2012. Optimization of antibiotic analysis in water by solid-phase extraction and high performance liquid chromatography–mass spectrometry/mass spectrometry. Anal. Chim. Acta 731, 32–39.

Zhou, J.L., Zhang, Z.L., Banks, E., Grover, D., Jiang, J.Q., 2009. Pharmaceutical residues in wastewater treatment works effluents and their impact on receiving river water. J. Hazard. Mater. 166, 655–661.

Zhou, L., Ying, G., Zhao, J., Yang, J., Wang, L., Yang, B., Liu, S., 2011. Trends in the occurrence of human and veterinary antibiotics in the sediments of the Yellow River, Hai River and Liao River in northern China. Environ. Pollut. 159, 1877–1885.

Zou, S.C., Xu, W.H., Zhang, R.J., Tang, J.H., Chen, Y.J., Zhang, G., 2011. Occurrence and distribution of antibiotics in coastal water of the Bohai Bay, China: impacts of river discharge and aquaculture activities. Estuar. Coast. Shelf Sci. 159, 2913–2920.