Antibiotics in the offshore waters of the Bohai Sea and the Yellow Sea in China: Occurrence, distribution and ecological risks

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ABSTRACT

The ocean is an important sink of land-based pollutants. Previous studies showed that serious antibiotic pollution occurred in the coastal waters, but limited studies focused on their presence in offshore waters. In this study, eleven antibiotics in three different categories were investigated in offshore waters of the Bohai Sea and the Yellow Sea in China. The results indicated that three antibiotics dehydration erythromycin, sulfamethoxazole and trimethoprim occurred throughout the offshore waters at concentrations of $0.10-16.6$ ng L^{-1} and they decreased exponentially from the rivers to the coastal and offshore waters. The other antibiotics all presented very low detection rates ($<10\%$) and concentrations (<0.51 ng L^{-1}). Although the concentrations were very low, risk assessment based on the calculated risk quotients (RQs) showed that sulfamethoxazole, dehydration erythromycin and clarithromycin at most of sampling sites posed medium or low ecological risks ($0.01 < RQ < 1$) to some sensitive aquatic organisms, including *Synechococcus leopoliensis* and *Pseudokirchneriella subcapitata*.

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1. Introduction

The residues of antibiotics are widely present in feces, medical waste, sewage treatment plants (STPs) and rivers due to their extensive and long-term usage in human therapies, animals, plant agriculture and aquaculture (Kummerer, 2009). Additionally, they tend to persist because of their continuous input (Daughton and Ternes, 1999). These residues can cause ecological harm in organisms and promote antibiotic resistance genes (ARGs), a newly emerging contaminant, in bacterial populations (Eguchi et al., 2004; Kummerer, 2004).

The ocean is an important sink of many terrestrial contaminants. Some recent studies showed that notable amounts of antibiotic residues were transported to coastal areas via riverine inputs (Jia et al., 2011; Zhang et al., 2012b; Zou et al., 2011) and STP effluents (Gulkowska et al., 2007; Minh et al., 2009) except for some residues that were used in mariculture (Jia et al., 2011). Although steep gradients from rivers (or STPs) to the sea were observed for most of the studied antibiotics due to dilution with seawater, many occurred widely in the coastal area, with some of the antibiotics causing high

ecological risks to some aquatic organisms. Because many antibiotics occurred in the coastal area, the question arises of whether and how much they are transported to the offshore area or the open sea, where no new inputs occur (apart from atmospheric deposition, which is not to be expected in the cases of the mostly polar or even ionic pharmaceuticals). However, knowledge on the presence of antibiotic contamination in these areas is very limited. Their detection in the offshore area or the open sea would confirm their ubiquitous character and could lead to new insights into their persistence. In this work, emphasis is placed upon antibiotics in the offshore waters in the Bohai Sea (BS) and the Yellow Sea (YS) of China based on our previous studies in the coastal water of the two seas.

The BS and YS were selected as the target study areas because their coasts (including four provinces and two municipalities under the direct jurisdiction of the Central Government) supported almost 25% of the Chinese population and provided 35% of the national GDP in 2008 (Lin et al., 2011). Dense populations, developed industries and agriculture, including animal husbandry and aquaculture, have produced a large amount of domestic, industrial, agricultural and aquacultural waste, which might contain antimicrobial agents, their degradation intermediates and antimicrobial-resistant bacteria (Ma et al., 2001). Additionally, the previous studies proved that these pollutants occurred widely in these

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wastes and were transported to the coastal areas, such as the three main bays of the BS (Bohai Bay, Laizhou Bay and Liaodong Bay), Jiaozhou Bay of the South YS and the bays around Yantai City in the North YS (Jia et al., 2011; Jiang et al., 2011; Luo et al., 2010, 2011; Wei et al., 2011; Zhang et al., 2012b; Zou et al., 2011). Moreover, the two seas are representative of two different types of bodies of water: the BS is a semi-enclosed sea and the YS is an open sea.

Similar to our previous studies, eleven antibiotics belonging to three groups, the macrolides (MLs), sulfonamides (SAs) and diaminopyrimidines (DMs), were selected as the target compounds in present study. The detailed information, including the physico-chemical properties and primary usage of the target compounds, are given in Supplementary Table S1. They are the most frequently prescribed antibiotics for human treatment and veterinary medicine in China. The antibiotics selected in this study were also widely present in the surface waters in Europe, the U.S.A., and China (Hirsch et al., 1999; Kolpin et al., 2002; McArdell et al., 2003; Xu et al., 2007; Zou et al., 2011).

The objectives of the present study were not only to comprehensively survey the occurrence and distribution of antibiotics in the offshore marine environment but also to assess their persistence and attenuation from the rivers to the offshore area. Meanwhile, their ecological risks to the aquatic organisms in the offshore marine environment were assessed using calculated risk quotients (RQs) (Hernando et al., 2006).

2. Materials and methods

2.1. Sampling stations and sample collection

The locations of sampling sites in the BS and YS are illustrated in Fig. 1A. A total of 62 surface water samples were collected during April 20–May 5, 2010 in one cruise on the "Dong Fang Hong 2" research vessel, including stations B10–B33 and FJ04–FJ09 in the BS and H1–H43, B2–B9 and B34–B48 in the YS. The station numbers in the study were discrete because they were the unified numbers of the cruise, but parts of the stations were selected in the study. Twenty-seven stations in the BS further included six sections: the Bohai Strait (BHS: B10–B17), the mouth of Liaodong Bay (LDM: B18–B23), the mouth of Bohai Bay (BHM: B24–B28), the mouth of the Laizhou Bay (LZM: B29–B33), the area adjacent to the estuary of the Yellow River (YRE: FJ04–FJ06) and the section near Longkou City (LK: FJ07–FJ09). Thirty-five stations in the YS further included two parts: the North YS (B05–B09 and B34–B48) and the South YS (H01–H43 and B02–B04). More information about the samples is given in Supplementary Table S2. All the samples were collected (approximately 0–50 cm below the water's surface) using a stainless steel bucket and were immediately transferred to a 5-L pre-cleaned amber glass bottle. The bottle was rinsed with the sample prior to sampling. The samples were kept at 4 °C in a cold storage room before further treatment and analysis in the laboratory.

2.2. Chemicals and standards

The selected compounds belonged to three different antibacterial families: MLs include erythromycin (ETM), spiramycin (SRM), azithromycin (AZM), clarithromycin (CTM) and roxithromycin (RTM); SAs consist of sulfadiazine (SDZ), sulfamethoxazole (SMX), sulfadimidine (SDM), sulfathiazole (STZ) and sulfacetamide (SAAM); DMs(trimethoprim (TMP)). The synergist TMP is often prescribed in combination with SAs and has similar properties to SAs, so it was usually grouped with the SAs
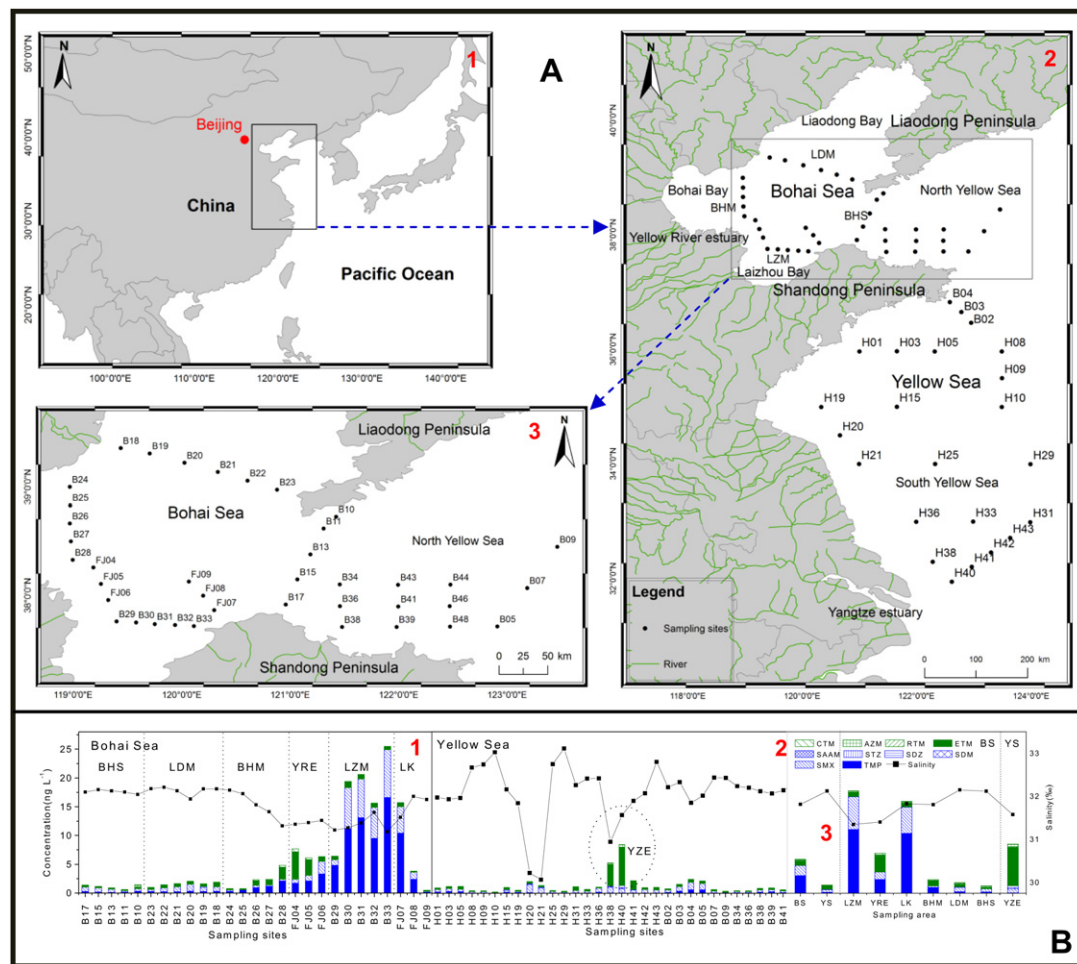
Fig. 1. A: Map of sampling sites in the Bohai Sea and the Yellow Sea, North China. B-1, 2: Antibiotic concentration and water salinity profiles in the Bohai Sea and the Yellow Sea. B-3: Regional statistic results of antibiotics and water salinity in the Bohai Sea and the Yellow Sea. The abbreviations BS and YS indicate the Bohai Sea and the Yellow Sea, respectively. BHM, LZM and LDM indicate the mouths of Bohai Bay, Laizhou Bay and Liaodong Bay, respectively, and BHS indicates the Bohai Strait. YRE and YZE indicate the areas adjacent to the estuaries of the Yellow River and the Yangtze River, respectively. LK indicates the section near Longkou City. ETM indicates dehydration erythromycin.

when discussing the results. All the target compounds were purchased from Sigma–Aldrich Co. (St. Louis, MO, USA). The $^{13}C_3$-caffeine solution was obtained from Cambridge Isotope Labs (1 mg mL^{-1} in methanol, USA) and used as surrogate standard. All the antibiotic compounds were dissolved in methanol and stored in a freezer. Erythromycin-H$_2$O (ETM-H$_2$O), a major degradation product of erythromycin, was obtained by acidification of erythromycin using the method described by McArdell et al. (2003).

Methanol (HPLC grade) was obtained from Merck (Darmstadt, Germany). Formic acid and ammonium acetate were purchased from CNW (Germany). Disodium edetate dihydrate (Na$_2$EDTA) was analytical grade and obtained from Tianjin Chemical (Tianjin, China). Ultra-pure water was prepared with a Milli-Q water purification system (Millipore, Bedford, Massachusetts, USA). Unless otherwise indicated, the chemicals used in the analysis were analytical grade or above.

2.3. Sample extraction and analysis

The antibiotics in the water were concentrated by the solid-phase extraction (SPE) using the Oasis HLB cartridge (500 mg, 6 mL, Waters Corporation, Milford, Massachusetts, USA) (Xu et al., 2007). Before extraction, a 5 L water sample was filtered through 0.7 μm glass fiber filters (GF/F, Whatman, Mainstone, England) and then acidified to pH = 3.0 with 3.0 mol L^{-1} H$_2$SO$_4$, followed by the addition of 0.2 g Na$_2$EDTA as the chelating agent and 100 ng of $^{13}C_3$-caffeine as the surrogate to monitor the recovery. The SPE cartridge was preconditioned. Each water sample was passed through the cartridge. The analytes were eluted with 2 mL × 3 of methanol, volume-reduced to approximately 20 μl, and then dissolved in 40% aqueous methanol to a final volume of 1.0 mL. The details of SPE process are shown in Supplementary S1.

The extracted samples were analyzed using high performance liquid chromatography–electrospray ionization tandem mass spectrometry (HPLC–ESI–MS–MS) with multiple reactions monitoring (MRM). The instrumental analysis method was also optimized based on our previous method (Zhang et al., 2012b) for the three antibiotic classes. The separation of the compounds was performed with Agilent 1200 series (Agilent, Palo Alto, USA) on an Agilent Zorbax XRD-C18 column (2.1 mm × 50 mm, 1.8 μm) with a guard column SecurityGuard™ C18 (4.0 mm × 3.0 mm). For mass spectrometric analysis, Agilent 6460 triple quadrupole mass spectrometer (Agilent, Palo Alto, USA) equipped with an electrospray ionization source in the positive mode (ESI+) was used to analyze the antibiotics. More conditions of liquid chromatography and mass spectrometry were described in Supplementary S2.

2.4. Quality analysis and quality control

A quantitative analysis of each compound was performed using HPLC–ESI–MS–MS in the MRM mode using two of the highest characteristic precursor ion/product ion transitions. Together with the retention times, the characteristic ions were used to ensure the correct peak assignment and peak purity. The $^{13}C_3$-caffeine was added as a surrogate standard to all samples prior to being filtered to compensate for the matrix effects during the analytical procedure. Although the use of multiple surrogate standards is preferred to enhance the accuracy of analysis of multiple compounds with different physicochemical properties, the unavailability of some labeled antibiotics and budget constraints precluded this approach. Because of such constraints, several recent studies similarly relied on the standards addition method plus a single internal standard to analyze multiple antibiotics.

The limits of quantification (LOQ) for each compound in the seawater obtained using the method described by Xu et al. (2007) ranged from 0.09 to 2.6 ng L^{-1} (Supplementary Table S4).

For the recovery experiments, the eleven target compounds were determined using the standards addition method, i.e., 5 L of filtered seawater fortified with 100 ng of target analytes was treated in the same procedure as the field samples. The recovery rates of these spiked antibiotics were 78–88% (Supplementary Table S4). The field and procedural blanks were treated as controls for possible contamination

in the laboratory and in the field sampling. Analysis of these blanks demonstrated that the extraction and sampling procedures were free of contamination. More detailed information about the quality analysis and quality control is shown in Supplementary S3.

2.5. Antibiotics attenuation model

Antibiotics attenuation (e.g., by adsorption, dilution, photolysis, hydrolysis and biodegradation) was assumed (and confirmed) to follow first-order kinetics. The attenuation coefficient (k) in the rivers was calculated as following by Luo et al. (2011):

$$k = \left(\frac{v}{L}\right)\ln\left(\frac{c_i}{c_I}\right) \tag{1}$$

where L is the distance from site i to site I; c_i and c_I are the antibiotic concentrations at sites i and I, respectively; and v is the average river velocity between the two sites. k was determined without the use of conservative tracers that would be needed to quantify dilution. Thus, k values represent overall attenuation coefficients that provide insight on the relative persistence and potential reach of different antibiotics (rather than specific degradation coefficients for use in predictive fate and transport models). This model was only applied to segments with a single pollution source, usually located in the tributaries. But in this study, the pollution source was not a single, the directions of sea currents were not stable and the velocities of the sea currents were difficult to be available. Therefore, we just talked about the correlation of antibiotics contents with the distance and the k was calculated as:

$$k = \left(\frac{1}{L}\right)\ln\left(\frac{c_i}{c_I}\right) \tag{2}$$

The first-order attenuation assumption was tested by assessing the goodness of fit of the data to the exponential decay model [$C = C_0 e^{-kL}$] as described in the Supplementary Table S7.

3. Results and discussion

3.1. Detection frequency and overall concentration levels of the target antibiotics

Ten antibiotics of the eleven target compounds except SRM were detected at least once in the offshore waters of the BS and YS. The results are shown in Fig. 1B and summarized in Table 1. All raw data are available in Supplementary Table S5. As shown in Table 1, three antibiotics (ETM-H$_2$O, SMX and TMP) were detected widely, with detection rates of more than 73%. Among the ten detected antibiotics, ETM-H$_2$O, which was the predominant form of ETM in the aquatic environment (McArdell et al., 2003), was the most frequently detected compound with the detection rate of 100%. SMX showed the second highest detection rate of 98%, followed by TMP with a detection rate of 73%. Except for the three predominant antibiotics aforementioned, the other antibiotics were detected sporadically, with detection rates of less than 10%.

Concentrations of all the detected antibiotics ranged from 0.10 ng L^{-1} to 16.6 ng L^{-1}. Among the ten detected antibiotics, the three predominant antibiotics also presented the highest concentrations. The mean and maximum concentrations of the three predominant antibiotics were from 0.69 to 1.4 ng L^{-1} and from 6.7

Table 1
Summary of results for the selected antibiotics in the Bohai Sea and the Yellow Sea ($n = 62$).

	TMP + Sulfonamides (ng L^{-1})						Macrolides (ng L^{-1})			
	TMP	SMX	SDM	SDZ	SAAM	STZ	ETM-H$_2$O	RTM	AZM	CTM
Mean[a]	1.4	1.0	0.01	0.01	0.003	0.02	0.69	0.09	0.06	0.07
SD[b]	3.4	1.7	0.02	0.05	0.02	0.03	1.1	0.06	0.05	0.07
Median	0.22	0.45	nd	nd	nd	nd	0.31	<LOQ	<LOQ	<LOQ
Min	<LOQ	<LOQ	nd	nd	nd	nd	0.13	nd	nd	nd
Max	16.6	8.3	0.16	0.36	0.12	0.17	6.7	0.26	0.39	0.51
Detected rate (%)	73	98	1.6	4.8	1.6	4.8	100	1.6	6.5	8.1

nd: not detected.
LOQ: limits of quantification.
[a] Mean and SD values were calculated using the measured values if above the LOQ, the half of LOQ if <LOQ or 0 if not detected.
[b] Standard deviation.

to 16.6 ng L^{-1}, respectively, which were obviously lower than those in the coastal water, such as the Bohai Bay (Zou et al., 2011), Laizhou Bay (Zhang et al., 2012b), Liaodong Bay (Jia et al., 2011), Victor Harbor (Minh et al., 2009), Hong Kong coasts (Gulkowska et al., 2007) and Belgian coastal harbors (Wille et al., 2010) (Supplementary Table S6). Compared with the three predominant antibiotics, the other antibiotics showed much lower concentrations. Their maximum concentration was just 0.51 ng L^{-1}, and their mean concentrations (from 0.003 to 0.09 ng L^{-1}) were all below their LOQs because their concentrations were all below their LOQs in more than 90% of the samples. The percentage of the three major antibiotics accounted for up to 75–100% of the total concentrations of the detected antibiotics.

It should be noted that the presence of the three main antibiotics throughout the BS and YS indicated that they are sufficiently persistent to withstand transformation on their way to and within the seas, where no new inputs occur (Weigel et al., 2002). In addition, the significantly different physico-chemical and biological conditions in marine aquatic systems from limnic ones may lead to a completely different behavior of organic chemicals like, in some cases, an enhancement of stability (Weigel et al., 2002).

3.2. Environmental distributions of the target antibiotics

When interpreting the pollution distributions in the BS and YS, the pollution sources, the distance away the coast, water exchange ability and main water currents in the seas should be kept in mind. BS was the most polluted sea in China. It received approximately 36% of the wastewater and 47% of the solid pollutants in China (Wang and Wang, 2007). Additionally, it was a large aquaculture base and fish farming base. Its water exchange ability was much poorer than that of the YS because it was a semi-enclosed inner sea whereas the YS was an open sea. For the water currents, they are basically characterized by the flow of Bohai Sea Coastal Current (BSCC), Yellow Sea Warm Current (YSWC) and Yellow Sea Coastal Current (YSCC) (Guo et al., 2006), which were shown in the Supplementary Fig. S1. YSWC is the only source of open ocean water into the BS, YS, and even East Sea of China. When YSWC hit the coast of the Bohai Bay, it changes into two directions, one to the north, another to the south, and also forms a circular current in the Bohai Bay and out from the Liazhou Bay.

In general, the distribution patterns of the total target antibiotics showed that their total concentration in the BS (5.9 ng L^{-1}) was notably higher than that in the YS (1.4 ng L^{-1}) (Fig. 1B-3). It may be due to the larger number of pollution sources and the poor water exchange ability of the BS. And the low salinity of the BS (31.8‰) compared with the YS (32.1‰) (Fig. 1B-3) can also support the statement, i.e., open ocean seawater increased salinity and dilute pollutants and fresh water runoff decreased salinity but brings in antibiotics. Among the detected antibiotics, TMP and SMX in the two seas presented the most obvious differences. The concentrations of TMP and SMX in the BS were 21 and 3 times, respectively, those in the YS, whereas the other antibiotics were at nearly comparable levels in the two seas.

There were much more detailed regional spatial distributions features in the two seas (Fig. 1B and Supplementary Fig. S2). One significant feature was that much more MLs were detected in the areas adjacent to the estuaries of both the Yangtze River (YZE) and Yellow River. Their concentrations were the highest in the sites closest to the estuaries (H40 and FJ04) and decreased along those transects from H40 to H42, H40 to H36, FJ04 to FJ06 and FJ04 to B25. This feature might be impacted by the inputs of the Yangtze River and Yellow River, which also can be proven by the homochronous salinity distribution, i.e., the salinity increased with the river inputs along the transects away the coast. And the salinity negatively

correlated to the concentrations of ETM-H$_2$O in those transects mentioned above ($r^2 > 0.9$, $P < 0.01$) (Supplementary Fig. S3). Among the MLs, CTM, RTM and AZM were predominantly used by humans, whereas ETM can be used to treat humans and animals. However, the linear regression analysis indicated that a significant linear correlation occurred between ETM-H$_2$O and AZM in the area near YZE ($r^2 = 0.97$, $P = 0.04$) and between ETM and CTM in the area near YRE ($r^2 = 0.95$, $P = 0.12$) (Supplementary Fig. S4). This correlation may indicate that ETM-H$_2$O is from same source (domestic sewage) and/or presents the same environmental behavior as CTM and AZM (Zhang et al., 2012a).

Another significant feature was that the total antibiotic concentrations in BS showed the following regional distribution feature: LZM (17.7 ng L^{-1}) > BHM (2.4 ng L^{-1}) > LDM (1.9 ng L^{-1}) > BHS (1.3 ng L^{-1}) (Fig. 1B-3). The Laizhou Bay contributed much more TMP and SMX to the BS. Their concentrations at sites from B30 to B33 of the LZM and its adjacent site FJ07 were much higher than those at the other sites. Moreover, the concentration of TMP decreased gradually along those transects (from FJ06 to FJ04 and from B28 to B24) off the Laizhou Bay, and the concentration of SMX also decreased along the transect from FJ06 to FJ04. These results imply that the TMP and SMX in a large sea area of the BS were impacted by the Laizhou Bay. In addition, the area impacted by TMP was larger than that by SMX, which may be because TMP presented a much higher concentration than SMX in the LZM. Considering the sea current YSWC, the antibiotics detected at sites in Laizhou Bay (the highest in this study) may come from sources of both runoffs in the Bohai Bay and the Laizhou Bay. But surely TMP and SMX are mainly from the Laizhou Bay because their mean concentrations in the whole Laizhou Bay were even higher than those in the coast area of the Bohai Bay (Fig. 2) (Zhang et al., 2012b; Zou et al., 2011).
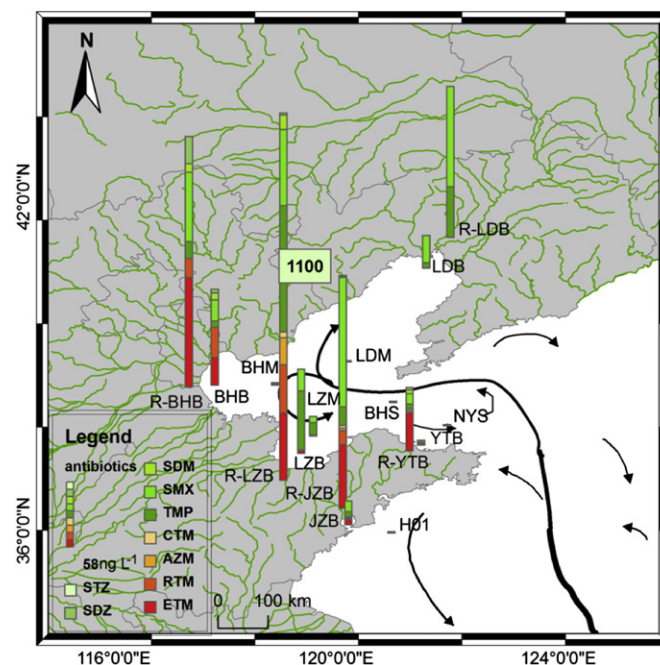

Fig. 2. Antibiotic concentration (mean) profiles in the rivers, coastal waters and offshore waters of the Bohai Sea. The abbreviation ETM indicates dehydration erythromycin. The abbreviation R indicates rivers adjacent to the sea. BHB, LZB, LDB, BHM, LZM, LDM, BHS, JZB, YTB and NYS indicate Bohai Bay, Laizhou Bay, Liaodong Bay, mouth of the Bohai Bay, mouth of the Laizhou Bay, mouth of the Liaodong Bay, Bohai Strait, Jiaozhou Bay, bays near Yantai City and North Yellow Sea, respectively. Some of the data in the figure are from Jia et al. (2011), Zhang (2011), Zhang et al. (2012a) and Zou et al. (2011).

Another characteristic was that the concentrations of most of the antibiotics decreased gradually along these transects off the coast. For example, steep gradients along the transects from FJ07 to FJ09, from B04 to B02, and from B39 to B43 were observed for the three major antibiotics due to dilution with clean seawater, sorption to particles, and/or degradation on their transport path, such as hydrolysis, photolysis, biodegradation, and so on.

The correlations between salinity and the antibiotic concentration in the some local area were discussed above. In the view of the more large area, inverse correlation also can be found between the salinity and the total antibiotic concentrations in the BS ($r^2 = 0.56$, $n = 27$, $P < 0.001$), the YS ($r^2 = 0.23$, $n = 35$, $P < 0.001$) and even the whole of the two seas ($r^2 = 0.24$, $n = 62$, $P < 0.001$) (Supplementary Fig. S5), suggesting that the input of fresh water enriched the antibiotics in the marine environment. Moreover, the relationship of salinity and antibiotic concentration in the seas can explain the distribution of antibiotics in the LZM, BHM and LDM to some extent. For example, the Laizhou Bay is the smallest in the three bays and the distance of the mouth to the coast is the shortest, so its mouth was impacted seriously by the coastal water (or river water), which was proved by the salinity in the mouth (lowest in the three bay mouths). Therefore, the antibiotic concentration in the mouth was the highest. The revise feature occurred in the Liaodong Bay, i.e., the distance of the LDM to the coast of Liaodong Bay was the longest, the salinity in the LDM was the highest while the antibiotic concentration in there was the lowest. Therefore, the antibiotic concentrations in LZM, BHM and LDM were related to the distance of the sampling area to the coast to some extent.

3.3. Transport of antibiotics from the rivers to the offshore waters

Fig. 2 demonstrates the distribution of antibiotics in the rivers discharging to sea, coast and offshore in some bays of the BS and the YS. As this figure shown, the antibiotic contents decreased obviously in the following order in the three bays of the BS: rivers > coast > bay mouth. The contents also decreased further in the BHS. In the bays of YS, the order was same: rivers > coast > offshore. The similar feature was also found from the sites near the STPs towards the outside of the shelter in the Victor Harbor (Minh et al., 2009). The phenomenon may clearly show the transport process of the antibiotics from the rivers to the offshore areas with the river inputs, sea currents and/or diffusion. Moreover, they attenuated sharply on the transport process because of the adsorption, dilution, photolysis, hydrolysis and/or biodegradation. The attenuation can even be described roughly by the exponential attenuation model $C = C_0 e^{-kL}$ mentioned in Section 2.5 (Supplementary Table S7), which also applied in the coastal waters of the Bohai Bay and the Jiaozhou Bay (Zhang, 2011). The attenuation rate coefficient (k values) indicated that ETM-H$_2$O (k: 0.052–0.069 km^{-1}) was the most prone to attenuation, followed by TMP (k: 0.041–0.051 km^{-1}) and SMX (k: 0.02–0.06 km^{-1}). The

same trend was observed in the coastal waters of the Bohai Bay and Jiaozhou Bay (Zhang, 2011). The sorption to particles and sediments (Li and Zhang, 2010; Tolls, 2001) and relatively shorter half-life (Xu, 2007) are two important mechanisms contributing to the relatively high attenuation capacity for ETM-H$_2$O. The lower k values of TMP and SMX indicated that the two antibiotics were more stable than ETM-H$_2$O on the one hand. On the other hand, the use of TMP and SMX in the mariculture may be another reason for their lower k values. However, the k values in this study were obviously lower than those in the coastal waters of the Bohai Bay and Jiaozhou Bay, which may demonstrate that these antibiotics attenuate slowly at trace concentrations and/or in the offshore environment because of their different physico-chemical and biological conditions.

3.4. Risk assessment

Given that some of the antibiotics occurred widely in the BS and YS, although at very low levels in the offshore areas, do they pose an ecological risk to aquatic organisms? The potential environmental risks of the detected antibiotics were assessed on the basis of the risk quotients (RQs), according to the European technical guidance document on risk assessment (TGD) (EC, 2003). The RQ value can be calculated through the predicted environmental concentration (PEC) or measured environmental concentration (MEC) divided by the predicted no-effect concentration (PNEC). According to the TGD, when only short-term/acute toxicity data EC50/LC50 are available, the calculation of PNEC is obtained from the EC50/LC50 divided by an assessment factor of 1000. Once the long-term/chronic NOEC values for one, two or three trophic levels are available, an assessment factor of 100, 50 or 10 is used (EC, 2003). In some studies, however, an assessment factor of 1000 was used for the long-term/chronic EC50/LC50 values, although the assessment factor reduces the degree of uncertainty in the extrapolation from the test data on a limited number of species compared with the real environment (Isidori et al., 2005). In this study, the acute or chronic toxicity data of the selected antibiotics on non-target organisms were collected from the literature and are shown in Supplementary Tables S8–S10. The PNEC values were also calculated based on the toxicity data and are shown in Table 2. Additionally, the RQ for each detected antibiotic was calculated using the MEC in the surface water of the studied area and the PNEC values shown in Table 2 (Fig. 3).

To better elucidate the risk levels, the ratios were classified into three risk levels: 0.01–0.1, low risk; 0.1–1, medium risk; and >1, high risk (Hernando et al., 2006). Three antibiotics, SMX, ETM-H$_2$O and CTM, posed at least a low risk to aquatic organisms both in the BS and YS (Fig. 3). All three of these antibiotics could pose as a medium risk to the relevant sensitive aquatic organisms (*Synechococcus leopoliensis*, *Pseudokirchneriella subcapitata* and *Pseudokirchneriella subcapitata*, respectively) in the BS. The proportions

Table 2
Aquatic toxicity data for the eight selected antibiotics and the most sensitive aquatic species.

Compound	Non-target organism	Toxicity data (mg L^{-1})	Toxicity	AF[a]	PNEC[b] (ng L^{-1})	Reference
TMP	*R. salina*	EC50 = 16	Acute	1000	16,000	(Lutzhoft et al., 1999)
SMX	*S. leopoliensis*	EC50 = 0.027	Acute	1000	27	(Ferrari et al., 2004)
SDM	*S. vacuolatus*	EC50 = 19.52	Chronic	1000	19,520	(Bialk-Bielinska et al., 2011)
SDZ	*S. capricornutum*	EC50 = 2.2	Acute	1000	2200	(Eguchi et al., 2004)
ETM-H$_2$O	*P. subcapitata*	EC50 = 0.02	Chronic	1000	20	(Isidori et al., 2005)
RTM	*P. subcapitata*	NOEC = 0.01	Chronic	100	100	(Yang et al., 2008)
CTM	*P. subcapitata*	EC50 = 0.002	Chronic	1000	2	(Isidori et al., 2005)
AZM	*Daphnia* sp.	EC50 > 120	Acute	1000	>120,000	(FDA-CDER, 1996)

NOEC: no observable effect concentration.
EC50: half maximal effective concentration.
[a] AF: Assessment factor.
[b] PNEC: Predicted no effect concentration.
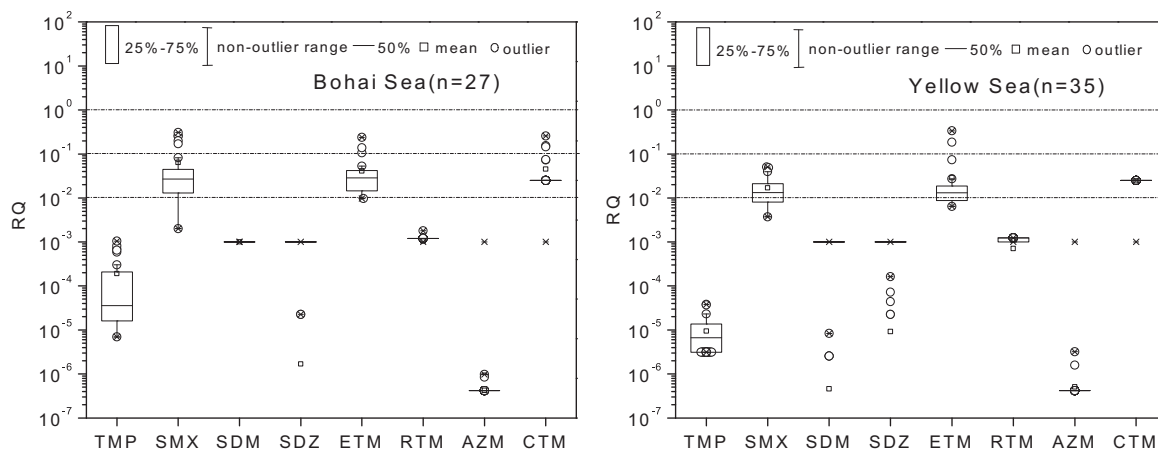
Fig. 3. Boxplots for the calculated risk quotients (RQs) for the eight antibiotics detected in the Bohai Sea and the Yellow Sea. The abbreviation ETM indicates dehydration erythromycin.

of the samples causing medium risk by SMX, ETM-H_2O and CTM in the BS were 19%, 11% and 11%, respectively. These samples were found mainly in the LZM and YRE (Supplementary Fig. S6). In the YS, however, only one antibiotic, ETM-H_2O, posed a medium risk to the aquatic organism in the two sites near the YZE (Supplementary Fig. S6). In most of the sampling sites of YS, the three antibiotics posed a low risk. The proportions were 69%, 63% and 97% for SMX, ETM-H_2O and CTM, respectively. It should be noted that the CTM levels in all the YS samples were below the LOQ or not detected. CTM levels below the LOQ had RQs between 0.01 and 0.1 when ½ LOQ was used to calculate the RQ. Therefore, the CTM in these sites could pose a potential low risk to *P. subcapitata*. Spatially, the antibiotics pose a higher risk in the sites near the coast relative to the offshore sites. For example, for ETM-H_2O and SMX, the sites with RQs below 0.01 were mainly those sites furthest from the coast, such as B09, H08, H09 and H10 (Supplementary Fig. S6). Although their concentrations were not very high, SMX, ETM-H_2O and CTM in these study areas, especially the areas near the coast, still showed relatively higher ecological risks to the relevant aquatic organisms. Similar results were reported in Laizhou Bay of the BS (Zhang et al., 2012b), Beibu Gulf of the South China Sea (Zheng et al., 2012) and in the surface waters of Germany (Kummerer and Henninger, 2003) and Korea (Lee et al., 2008). Thus, monitoring the detection of antibiotics in our surface waters and regulating their abuse is a worthwhile effort.

However, it should be noted that the risk assessment above has some limitations, as we could not collect all the toxicity data and the mixture toxicity of the compounds was not considered.

4. Conclusions

Eleven selected antibiotics belonging to three categories were investigated in a wide range of offshore surface water from the BS and the YS of China. Three antibiotics, ETM-H_2O, SMX and TMP, occurred widely in the offshore water of the two seas with the concentrations of 0.10–16.7 ng L^{-1}, although they were obviously lower than those in the coastal water reported by the previous studies. In terms of their regional distribution in the two seas, higher concentrations were detected in the BS due to the poor water exchange ability and the larger amount of sewage discharged into the sea. Higher concentrations of macrolides mostly occurred in the estuaries of the Yellow River and Yangtze River, indicating that riverine inputs were the primary source of macrolides in the seas. Inverse correlation can be found between salinity and the total

antibiotic concentrations in the two seas, suggesting that the input of fresh water enriched the antibiotics in the marine environment. Moreover, the exponential decrease of the antibiotic concentrations from the rivers to the offshore areas also confirmed their transport process from river to the ocean. Although these antibiotics attenuated exponentially on their transport process, they were detected in the offshore area approximately 400 km away from the coast, which indicated that they are sufficiently persistent in the seas. In addition, although the concentrations in the offshore waters were not very high, risk assessment based on the calculated risk quotient (RQ) showed that SMX, ETM-H_2O and CTM at most of sampling sites can pose medium or low ecological risks ($0.01 < RQ < 1$) to the most sensitive aquatic organisms, such as *Synechococcus leopoliensis* and *Pseudokirchneriella subcapitata*.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.envpol.2012.11.008.

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