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Occurrence and distribution of antibiotics in mariculture farms, estuaries and the coast of the Beibu Gulf, China: Bioconcentration and diet safety of seafood

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ABSTRACT

The occurrence, distribution, bioconcentration and diet safety via seafood consumption of 19 antibiotics were investigated in eight closed mariculture ponds, four estuaries, two nearshore areas and one offshore area from the Beibu Gulf. Seventeen, 16, 15 and 7 antibiotics were detected at total concentrations of 43.2 − 885 ng L^{-1}, 22.4 − 118 ng L^{-1}, 22.7 − 24.5 ng L^{-1}, and 1.81–3.23 ng L^{-1} in the water of the above different areas, respectively. This indicates that the mariculture ponds are important sources of antibiotic pollution on the coast of the Beibu Gulf. Ten antibiotics were detected in feed samples with concentrations ranging from 0.03 to 95.4 ng g^{-1}, demonstrating the presence of antibiotics in the feed and/or residual antibiotics in the raw material of the feed. The field bioconcentration factors (BCFs) of the antibiotics calculated in different culture organisms ranged from 0.55 to 10,774 L kg^{-1}. The estimated daily intakes (EDIs) of sulphonamides, fluoroquinolones, macrolides and chloramphenicols via aquatic products were 19.8–105, 33.7–178, 34.9–186 and 6.9–37.1 ng d^{-1}, respectively. According to the acceptable daily intakes (ADIs) and maximum residue limits (MRLs) proposed by different organisations, these aquatic products (shrimp, crab and oyster) reached the standard of safe consumption and could not pose a health risk to humans. However, a potential elevated risk to humans may remain because of the occurrence of multiple antibiotics in the cultured organisms, particularly for sensitive populations, such as pregnant women, the elderly and children.

1. Introduction

Antibiotics, as a type of effective antimicrobial agent, are widely used for bacterial disease prophylaxis and treatment in humans and animals and also as feed additives to promote growth in husbandry and aquaculture. China leads the world in antibiotic production and consumption (Chen et al., 2015a; Luo et al., 2011). It has been estimated that the annual production of antibiotics is approximately 248,000 t, of which 90% is used for humans (48%) and to treat animals (42%), and the remaining 10% is exported (Chen et al., 2015a). In recent years, the residues of antibiotics have been frequently found in water (Ma et al., 2015; Schwartz et al., 2003; Xu et al., 2007; Yan et al., 2013; Zhang et al., 2012a), sediments (Zhou et al., 2016), soils (Martinez-Carballo et al., 2007), suspended particles (Zhang et al., 2017; Zhou et al., 2013), faeces (Zhou et al., 2013)and biota samples (Chen et al., 2015b; Li et al., 2012). Antibiotics can cause toxic effects to aquatic and terrestrial organisms (Kim et al., 2007; Kotzerke et al., 2008) and can also bioaccumulate in organism. In addition, their continuous application can promote antibiotic resistance genes (ARGs) in bacterial populations (Eguchi et al., 2004; Su et al., 2012).

Various antibiotics are widely used in aquaculture to prevent disease, improve feed utilization and promote animal growth. However, only 20~30% is absorbed and utilized by aquaculture products. The remaining antibiotics persist in the aquatic environment and are discharged into the surrounding water, or they are deposited in the sediments of aquaculture ponds (Brooks et al., 2005; Mimeault et al., 2005; Samueisen, 1989; Schwaiger et al., 2004). This not only affects aquatic ecological environment but also leads to their concentrations in aquatic products over national and international food safety standards. Therefore, antibiotic residues in the aquaculture environment should be paid much attention due to their potential risk to human consumer.

The Beibu Gulf, located in the northwest of the South China Sea, is

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surrounded by Leizhou Peninsula, Qiongzhou Strait, Hainan Island, Vietnam and Guangxi Zhuang Autonomous Region. It covers an area of 1.3×10^5 km^2 and is a semi-closed gulf with an average depth of approximately 38 m (Chen et al., 2009). The climate around the gulf is subtropical and monsoonal (Chen et al., 2009). As an important mariculture base, the Beibu Gulf is one of the four famous fishing grounds in China. With a wide shallow sea and mudflat, it is suitable for the breeding and growth of multiple marine organisms. The mariculture area and production of Guangxi Province in 2012 were 530 km^2 and 977 thousand tons, respectively (Huang, 2013). The catch in the Beibu Gulf reached 857 thousand tons in 2012 (Zou et al., 2013). There are two main culture types based on different food sources: (1) fish, shrimp and crab culture, which depend on artificial feed and are usually cultured in closed high level ponds; and (2) shellfish culture, which ingest natural phytoplankton and are usually cultured in open estuaries and shallow seas. The fish, shrimp and crab culture area was 227 km^2, and production was 254 thousand tons. The shellfish culture area was 303 km^2, and production was 723 thousand tons (Huang, 2013). Organic matter and nutrients can be discharged into the gulf with water flow. Rapidly developing mariculture in the Beibu Gulf has caused marine antibiotic pollution. A previous study in 2010 showed that certain antibiotics were detected in seawater from the coast, estuary and the vicinity of an aquaculture farm in the gulf, with the highest concentration ranging from 0.53 to 51 ng L^{-1} (Zheng et al., 2012). Moreover, a higher intensity of aquaculture activities could contribute to increasing levels of antibiotics in the environment (Zheng et al., 2012). However, that study only referred to a few seawater samples in the vicinity of mariculture farms. Questions about the occurrence, distribution, bioaccumulation, food exposure risks and environmental impact of antibiotics in mariculture farms in a typical subtropical gulf were not resolved. Therefore, a comprehensive study is needed to resolve these questions. The objectives of this study are to: (1) select two typical culture models (high-place culture ponds and bamboo raft culture in an estuary) and natural sea areas (estuaries, nearshore and offshore areas); (2) investigate the occurrence and distribution of 19 antibiotics belonging to four classes among water, sediment, marine product and feed samples in mariculture farms on the marine environment; (3) calculate the bioaccumulation factors of antibiotics in different culture organisms; (4) estimate daily intakes of the marine products and evaluate the human risk of dietary exposure.

2. Materials and methods

2.1. Standards and reagents

The 19 target compounds belong to the following four different antibacterial groups: 1) 8 sulphonamides (SAs), including sulfacetamide (SAAM), sulfadiazine (SDZ), sulfamethoxazole (SMX), sulfathiazole (STZ), sulfamethazine (SMZ), sulfapyridine (SPD), sulfadimethoxine (SDM), and trimethoprim (TMP); 2) 5 fluoroquinolones (FQs), including norfloxacin (NOX), ciprofloxacin (CIX), enrofluxacin (ENR), ofloxacin (OFX), and enoxacin (ENX); 3) 4 macrolides (MLs), including erythromycin (ETM), clarithromycin (CTM), azithromycin (AZM), and roxithromycin (RTM); and 4) 2 chloramphenicols (CAPs), including florfenicol (FF) and chloramphenicol (CAP). The 19 high purity standards noted above and 4 isotope-labelled compounds used as surrogate standards ($^{13}C_3$-Caffeine, ^{13}C, D_3- ETM, $^{13}C_6$-SMX, D_5-NOX) were purchased from different manufacturers (Supplementary Table S1). Additionally, information on standards and reagents is summarised in the Supplementary information S1.

2.2. Sample collection

Seventeen sampling sites were investigated in this study (Fig. 1), including seven high-place shrimp culture ponds (01 P, 02 P, 04 P, 05 P, 07 P, 10 P and 12 P), one mudskipper culture pond (13 P), four estuaries (06E, 08E, 09E and 11E), two nearshore sites (03 N and 14 N), and three offshore sites (15 O, 16 O and 17 O) near Weizhou Island. All shrimp ponds cultured *Litopenaenus vannamei*, the 01 P, 04 P and 07 P ponds polycultured crabs (*Scylla paramamosain*). The estuaries are main oyster (*Crassostrea rivularis Gould*) culture areas. The offshore sites were selected as background. All samples (water, sediment, marine product and feed) were collected from the 17 sites in October 2015. Additionally, relevant sampling information is given in Tables S2 and S3.

All 17 water samples were collected using a stainless-steel bucket and were immediately poured into a 1-litre pre-cleaned amber glass bottle. Twelve sediment samples, fifteen biota samples and three feed samples were collected into sealed polyethylene bags. The detailed information on the sampling sites for sediment, biota and feed is summarised in Supplementary Table S2. All samples were stored at 4 °C during transport to the laboratory. The water samples were processed within 48 h. The weight and length of each biota sample were recorded. Then, the shrimp and oyster muscles were dissected with medical operation scissors and homogenized with agate mortar. The crab samples were divided into three parts (CL: crab leg muscle, CP: crab pereion muscle, and CR: crab roe) and homogenized. The sediment samples were freeze dried using freeze drier, ground and homogenized with agate mortar before passing through 80 mesh stainless steel sieve. All prepared biota and sediment samples were stored at −20 °C in a refrigerator prior to extraction.

2.3. Sample extraction and instrumental analysis

The extraction and instrumental analysis of water, sediment, feed and biota samples was performed as previously described (Chen et al., 2015b; Xu et al., 2007; Zhou et al., 2012). The water sample (1 L) was extracted with an Oasis hydrophile-lipophile balance (HLB) cartridge (6 mL, 500 mg), whereas the sediment and feed (5 g dry weight) were extracted by ultrasonic-assisted extraction with acetonitrile and citric acid buffer (pH = 3), followed by an enrichment and clean-up step with solid-phase extraction using strong anion exchange-HLB (SAX-HLB) cartridges in tandem. The biota samples (5 g wet weight) were extracted with methanol/water-0.1 M acetic acid (50:50, v/v) by ultrasonication with SAX/PSA-HLB cartridges in tandem. Additional details are shown in Supplementary S2.

Samples were analysed using an ultra-performance liquid chromatography–electrospray-ionisation tandem mass spectrometry (UPLC–ESI-MS–MS, Agilent UPLC 1290 tandem 6460 QQQ) with multiple-reaction monitoring (MRM). Separation of the target compounds was achieved on an Agilent Zorbax RRHD Eclipse Plus C18 column (2.1 mm × 100 mm, 1.8 μm) at 40 °C. Methanol (B) and high-purity water containing 5 mmol L^{-1} ammonium acetate aqueous solution with 0.1% formic acid (A) were the mobile phases, and the injection volume was 5.0 μL. A binary gradient at a flow rate of 0.3 mL/min was applied. The mobile gradient is shown in Table S5.

2.4. Quality assurance and quality control

A quantitative analysis of each compound was performed using UPLC-ESI-MS-MS with MRM mode using one or two of the highest characteristic precursor ion/product ion transitions. Together with the retention times, the characteristic ions were used to ensure correct peak assignment and peak purity. A known amount of $^{13}C_3$-CAF, ^{13}C,D_3-ETM, $^{13}C_6$- SMX and D_5- NOX were added as surrogate standards to each sample prior to monitoring the analytical recovery efficiency before extraction, and the concentrations of all types of samples were corrected based on recoveries. The respective recoveries of $^{13}C_3$-CAF, $^{13}C_6$-SMX, D_5-NOX, and ^{13}C, D_3-ETM were 80–92%, 82–106%, 62–87% and 72–90% in water samples, 67–92%, 70–90%, 60–81% and 65–82% in sediment samples and 65–89%, 67–85%, 70–93% and 63–80% in biological samples, respectively. Instrumental detection limits (IDLs) were defined as 3 times the signal-to-noise (S/N) ratio, and