Occurrence and risk assessment of antibiotics in river water in Hong Kong

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ABSTRACT

The occurrence and distribution of six typical antibiotics in the main rivers of Hong Kong were investigated using high-performance liquid chromatography electrospray ionization tandem mass spectrometry (HPLC-ES-MS/MS). The results revealed that the antibiotics were widely distributed in the area studied. Of the target antibiotics, ofloxacin was the most frequently detected in the rivers, with a detection rate of 69.6% and a median concentration of 0.7 ng/L. Sulfadimidine (n.d.–580.4 ng/L) and doxycycline (n.d.–82.2 ng/L), with detection frequencies of 65.2% and 30.4%, respectively, were found at the same level as in rivers in North America, Spain, France, Australia, and in the Yangtze and Pearl Rivers of China, while the other target antibiotics were found at lower levels. According to the ratios of the measured environmental concentration to the predicted no-effect concentration, ofloxacin and doxycycline could present a medium to low ecological risk to algae, while sulfonamides posed no obvious ecological risk to the relevant aquatic organisms (algae, Daphnia magna, and fish). A high detection rate of antibiotics occurred in densely populated areas, revealing that population activities might be greatly contributing to the increasing levels of antibiotics in the area. Thus, the residues of antibiotics present in the waters of Hong Kong need to be closely monitored.

1. Introduction

Antibiotics have been widely used as human and veterinary medicines and as growth promoters in agriculture and aquaculture (Kümmerer, 2009). The rate of antibiotic use has increased rapidly in recent years (Cabello, 2006; Zhang et al., 2012). Due to incomplete absorption and metabolism in the target organisms, a considerable fraction is released into the environment (Sarmah et al., 2006) through a variety of routes, such as sewage effluent, surface runoff, agricultural activities, and animal waste discharge (Boxall et al., 2002; Miao et al., 2004; Davis et al., 2006; Kümmerer 2009). Continuous input, together with the numerous pollution sources, have led to the pseudo-persistent characteristics of antibiotics in the environment (Daughton and Ternes, 1999; Richardson et al., 2005; Khetan and Collins, 2007). Some of the antibiotics, such as sulfonamides which are hydrolytically stable, hard to degrade in water and easy to be transported, are found in larger amounts in groundwater and surface water (Boxall et al., 2002; Stob et al., 2007). Antibiotics, together with their degradation and metabolic products have been detected in many environmental compartments, including surface water (Tang et al., 2015), river water (Tamtam et al., 2008; Jiang et al., 2011; Li et al., 2015), seawater (Minh et al., 2009; Zou et al., 2011), municipal sewage (Li et al., 2009; Lindberg et al., 2010), sludge (Göbel el al., 2005; Lindberg et al., 2010), soil (Christian et al., 2003), sediment (Pé et al., 2006), groundwater (Standley et al., 2008; Fick et al., 2009; Teijon et al., 2010; López-Serna et al., 2013), and even drinking water (Yiruhan et al., 2010).

In recent years, there has been increasing concern about the risk of antibiotic residues in the aquatic environment. Antibiotic contamination can cause hazardous effects in aquatic organisms and is a potential risk to the food chain, even at low concentrations (Gao et al., 2012; Bouki et al., 2013). In addition, the overuse and massive discharge of antibiotics has led to the emergence, development and spread of antibiotic resistance genes (ARGs), which have been classified as one of the three biggest threats to public health in the twenty-first century by the World Health...
Organization (Rico et al., 2012; Bouki et al., 2013; Marti et al., 2014; Skariyachan et al., 2015). The ARGs have been illustrated in river water, wastewater and recycled water, might be a serious threat to the ecosystem and human health (Chen and Zhang, 2013). Therefore, local and large-scale studies are urgently needed to fully assess their occurrence.

Hong Kong is in the Pearl River Delta located in Southern China, one of the most densely populated areas in the world and one of the most economically developed regions in China. Antibiotics, including sulfonamides and fluoroquinolones have been frequently detected in this region. Of particular concern is Victoria Harbor in the South China Sea, which received a total input of up to 14.4 kg per day of antibiotics from seven sewage plants (Richardson et al., 2005; Xu et al., 2007a, 2007b; Yang et al., 2011; Huang et al., 2012; Peng et al., 2012). Considering that Hong Kong has a long coastline with the South China Sea and the marine environment is a major receptacle of antibiotic residue (Zhang et al., 2013b) via sewage effluent (Xu et al., 2007a, 2007b) or riverine input (Na et al., 2013; Zhang et al., 2013c), antibiotics in this area could pose an ecological risk to the marine environment, in addition to the local environment.

Nevertheless, previous surveys of antibiotics have mainly focused on the Pearl River region in Guangzhou or on individual areas of Hong Kong (Xu et al., 2007a, 2007b; Minh et al., 2009; Yang et al., 2011). Data regarding the contamination and distribution of antibiotics in the river water of the entire region of Hong Kong are limited. To develop a better understanding of antibiotic pollution, a region-wide survey across Hong Kong was conducted for the first time. Overall, 25 sites covering the main rivers in Hong Kong were selected and analyzed for the presence of the three most commonly used classes of antibiotic, tetracyclines, fluoroquinolones, and sulfonamides, using high-performance liquid chromatography electrospray ionization tandem mass spectrometry (HPLC-ES-MS/MS) technology. Six antibiotics were selected for this study based on the known antibiotics used in Hong Kong, including doxycycline (DO), a tetracycline; ofloxacin (OFL) a fluoroquinolone; and sulfadiazine (SD), sulfamethoxazole (SMX), sulfadimidine (SMZ), and sulfapyridine (SP) belonging to the sulfonamides. The results would have significant implications to understand the occurrence of antibiotics in the Hong Kong area. In addition, an environmental risk assessment was conducted, to provide information for future decision-making.

2. Materials and methods

2.1. Chemicals

All of the antibiotic standards doxycycline (DO), ofloxacin (OFL), sulfadiazine (SD), sulfamethoxazole (SMX), sulfadimidine (SMZ), sulfapyridine (SP) and the internal standard (simeton), were purchased from Sigma-Aldrich (Missouri, USA). The chemicals were of > 98% purity and were used directly in the experiments. Acetonitrile (ACN) and methanol were HPLC-grade and were obtained from Fisher Scientific (USA), formic acid (FA, 99%) was purchased from Fluka (USA) and NH₄AC was purchased from Sinopharm Chemical Reagent Co., Ltd., China. Ultra-pure water was prepared with a Milli-Q water purification system (Millipore, Billerica, MA, USA). Standard antibiotic stock solutions with concentrations of up to 500 μg/mL were prepared in methanol (HPLC grade) and stored at −20 °C. Working solutions of different concentrations were prepared before chemical analysis.

2.2. Sampling

The sampling was conducted in September 2014 in Hong Kong, from 23 river surface water samples and two sewage samples.
of target analytes and MRM parameters used for qualitative and quantitative determination is presented in the support information in Table S2.

2.4. Quality assurance/quality control

The concentrations of the six antibiotics were calculated using the internal standard method. A quality control sample (1 μg mL⁻¹ standard) was run every five injections, followed by a blank sample. A calibration curve consisting of a seven step gradient of the standard solutions (1, 5, 10, 20, 50, 100, 200 μg mL⁻¹) was used. For each set of samples, the procedural blank was run to check for background contamination and system performance of the analysis procedure. The determination coefficients (R²) of the regression curves ranged from 0.992 to 0.999. The spiking recovery rates of the six antibiotics ranged from 61% to 89%, while their relative standard deviations for seven replicates ranged from 2.6% to 10.2%. The limits of quantification (LOQs) determined as the concentrations with a signal to noise ratio of 10 were estimated to be between 0.05 and 0.2, while the limits of detection (LODs) with a signal to noise ratio of three were between 0.02 and 0.08 ng/L.

2.5. Risk characterization

In order to evaluate the adverse ecological effects of antibiotics on the aquatic ecosystem, the environmental risks of the target antibiotics to aquatic species were assessed on the basis of the risk quotients (RQs). According to European Technical Guidance Document on risk assessment (EC, 2003), the RQ is usually calculated from the predicted or measured environmental concentration (MEC) and the predicted no-effect concentration (PNEC) obtained from EC50 or LC50 divided by assessment factor from acute toxicity data, or from chronic no observed effect concentration (NOEC) values divided by assessment factor. The risk was classified into high risk (RQs > 1), medium risk (0.1 < RQs < 1), and low risk (0.01 < RQs < 0.1) (Xu et al., 2013).

3. Results and discussion

3.1. Occurrence of antibiotics in water

A summary of the concentrations and detection frequencies of the six antibiotics in the river and sewage water is presented in Table 1. All target antibiotics were detected in the river water, indicating their ubiquitous occurrence in the rivers in Hong Kong. The total concentrations detected ranged from the LOQ to 580.4 ng/L in the river water and from the LOQ to 361.4 ng/L in the sewage water. Their median concentrations decreased in the order of DO (n.d. – 82.2 ng/L) in the river waters are higher
than other target antibiotics in most of the sampling sites in the rivers: SMX (n.d–3.1 ng/L), SD (n.d–14.8 ng/L), SP (n.d–3.2 ng/L) and OFL (n.d–4.3 ng/L). In the sewage influent the concentrations of the antibiotics were generally one to two orders of magnitude higher than those detected in the river water. However, at some sampling sites the concentrations of SMZ and DO in the river water were higher than in the sewage influent. These sites were mainly in two regions, KT3–KT6 of the Kam Tin River and SY3, SS1, and NT1 of the Shek Shuang River area, which were densely populated areas, suggesting that high intensity human activity might contribute to the increasing levels of antibiotics, some of which are approved by other researcher’s work that the levels and distribution of antibiotics in surface water were positively correlated with population densities (Xue et al., 2013).

Sulfonamides are hydrolytically stable and do not easily degrade in water (Stoob et al., 2007). They are likely to be transported to groundwater or surface water due to their high water solubility and weak sorption (Boxall et al., 2002). The levels of the sulfonamides detected were compared to the levels found in other countries. The concentration of SMZ in the river water (n.d–580.4 ng/L) was the same as in the Huangpu River (2.1–623.3 ng/L, Jiang et al., 2011; 19.9–389.4 ng/L, Chen and Zhou, 2014), urban surface waters of Beijing in China (n.d–123 ng/L, Li et al., 2015) and Pearl Rivers in China (n.d–1080 ng/L, Yang et al., 2011), and slightly higher than in the Yangtze Estuary in China (0.53–89.1 ng/L, Yan et al., 2013), the Ebro River in Spain (2.5–65.2 ng/L, Garcia-Galán et al., 2011), and stream water in North America (n.d–120 ng/L, Kolpin et al., 2002). The concentrations of the other target sulfonamides, including SMX (n.d–3.1 ng/L), SD (n.d–14.8 ng/L), and SP (n.d–3.2 ng/L), were lower than in other river waters in China and in countries such as Beijing (China) (Li et al., 2015), North America (Kolpin et al., 2002), Spain (Garcia-Galán et al., 2011; Boleda et al., 2013; Mendoza et al., 2015), France (Tamtam et al., 2008), and Australia (Watkinson et al., 2009) (Table 2).

The target antibiotic DO has a broad-spectrum antimicrobial activity and is widely used in animal therapy in China. The concentrations of DO (n.d–82.2 ng/L) were found to be comparable to those in the Huangpu River (n.d–113.9 ng/L, Jiang et al., 2011; n.d–54.3 ng/L, Chen and Zhou, 2014), Chaohu in China (n.d–42.3 ng/L, Tang et al., 2015) and in stream water in North America (n.d–110 ng/L, Kolpin et al., 2002), but were higher than those in the Ebro River (2.5–65.2 ng/L, Garcia-Galán et al., 2011), the Yangtze River (n.d–2.4 ng/L, Yan et al., 2013), and the Pearl River (0.19–13.69 ng/L, Yang et al., 2011). DO has a low degradation potential in the environment and is easily disposed of in soil or sediment (Kolpin et al., 2002; Andreozzi et al., 2003). Due to its high water solubility, it is likely to be transported into the groundwater by flowing water (Werner et al., 2006).

OFL is often detected in a variety of environmental samples (Zhang et al., 2013a; Guerra et al., 2014). The concentration of OFL in river water (n.d–4.3 ng/L) was lower compared to the Yellow River in China (n.d–129 ng/L, Xu et al., 2009), the Pearl River in China (n.d–80 ng/L, Yang et al., 2011), urban surface waters of Beijing in China (0.3–990 ng/L, Li et al., 2015) and the Seine River in France (n.d–55 ng/L, Tamtam et al., 2008). OFL was often detected in sewage effluents at up to 11 μg/L (Van Doorslaer et al., 2014; Zhang et al., 2013a). For the sewage water in the present study, OFL was detected to be as high as 135.9 ng/L in effluent. The possible reason is that it does not undergo biodegradation and has a high adsorption affinity, resulting in its persistence in the

### Table 1
Antibiotics concentrations detected in river water in Hong Kong (ng/L).

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Abbr.</th>
<th>CAS</th>
<th>River water</th>
<th>Sewage effluent</th>
<th>Sewage influent</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Freq.a</td>
<td>Meanb</td>
<td>Med.c</td>
</tr>
<tr>
<td>sulfadimidine</td>
<td>SMZ</td>
<td>57-68-1</td>
<td>65.2</td>
<td>83.4</td>
<td>16.5</td>
</tr>
<tr>
<td>sulfamethoxazole</td>
<td>SMX</td>
<td>723-46-6</td>
<td>47.8</td>
<td>1.2</td>
<td>1.1</td>
</tr>
<tr>
<td>sulfadiazine</td>
<td>SD</td>
<td>68-35-9</td>
<td>43.5</td>
<td>2.5</td>
<td>1.1</td>
</tr>
<tr>
<td>sulphapyridine</td>
<td>SP</td>
<td>144-83-2</td>
<td>60.9</td>
<td>1.15</td>
<td>0.9</td>
</tr>
<tr>
<td>doxycycline</td>
<td>DO</td>
<td>64-75-5</td>
<td>30.4</td>
<td>27.0</td>
<td>17.9</td>
</tr>
<tr>
<td>ofloxacin</td>
<td>OFL</td>
<td>82419-36-1</td>
<td>69.6</td>
<td>1.14</td>
<td>0.7</td>
</tr>
</tbody>
</table>

|                |       |              |             |                 |                 |                 |       |
|                |       |              | Freq.a      | Meanb          | Med.c           | Max.d          |       |
|                |       |              |             |                 |                 |                 |       |

a Frequency (%) of detection of each antibiotic in all samples.  
b Mean value of antibiotic concentrations (ng/L). Antibiotic concentrations below the limits of quantification (LOQ) were excluded from the calculations.  
c Median concentration (ng/L).  
d Maximum concentration (ng/L).
environment (Jones et al., 2005; Rosendahl et al., 2012; Van Doorslaer et al., 2014).

In general, the concentrations of some of the sulfonamides and of OFL in the Hong Kong surface waters were lower than in the Pearl River of Guangzhou, which is adjacent to Hong Kong, and lower than those in countries such as North America, Spain, France, and Australia (Kolpin et al., 2002; Tamtam et al., 2008; Watkinson et al., 2009; Doorslaer et al., 2014).

### 3.2. Environmental risk assessment

In this study, the MECs and the PNECs of each antibiotic for the algae, *daphnia magna* and fish were used to conduct a risk assessment and to calculate the RQs. In this study, the PNEC values of the target antibiotics were collected from the literature, which were based on the data on the acute or chronic toxicity to aquatic organisms, the details of toxicity data and PNECs were shown in the supporting information (Table S3).

The calculated RQs of the highest RQs of antibiotics for three aquatic organisms (*algae, daphnia magna* and fish) are shown in Table S4. Obviously, *daphnia magna* and fish are not likely at risk, because all their RQs are far lower than 0.01. However, algae are sensitive species to antibiotics in the aquatic environment (Fig. 3), and the sensitivity has been confirmed by other researchers (Halling-Sørensen et al., 2000; Ando et al., 2007; Li et al., 2015).

In Fig. 3, the risk assessment showed that OFL and DO presented a medium to low ecological risk to algae in river water in this area. For the sulfonamides detected, only SMZ posed a low ecological risk to algae in the KT3 and KT4 sampling sites, while SMZ, SMX, SD, and SP caused no risk to algae in the other sampling sites of the river. Overall, 26.1% of the river water samples posed a medium risk, while 39.1% posed a low risk. In the case of the sewage water, the influent (SW2) and effluent (SW1) both posed a high ecological risk due to the OFL present.

Compared with other studies, such as those in Laizhou Bay in east China (Zhang et al., 2012) and in Korea (Lee et al., 2008), where SMX and OFL levels were shown to pose relatively high ecological risks, we found that SMX posed a low ecological risk to aquatic microorganisms in 4% of the samples, while OFL posed a medium to high ecological risk in 24% of the samples.

Although the concentrations of the target antibiotics in the environmental samples were quite low, due to their continuous release, their potential to resist degradation, and their ability to absorb to soil or sediment, which can lead to contamination of groundwater and surface water, the presence of these pseudo-persistent compounds in the environment can be toxic to aquatic organisms (Boxall et al., 2003; Robinson et al., 2005). Moreover, the development and spread of ARGs is also a threat to the ecosystem and human health, and the ARGs will be transferred among different bacteria by various mechanisms and result in antibiotic resistance in the aquatic ecosystems (Marti et al., 2014; Skarjyachan et al., 2015). Thus, the environmental risk of these antibiotics needs to be evaluated to understand their adverse effects.
4. Conclusions

A regional extent survey was conducted to evaluate antibiotic pollution in the main rivers of Hong Kong. The results revealed the wide distribution of antibiotics in this area. SMZ and DO were detected at the same level as in rivers in North America, Spain, France, Australia, and in the Yangtze and Pearl Rivers of China, while the other target antibiotics were found at lower levels. An environmental risk assessment revealed that OFL and DO could present a medium to low ecological risk to relevant aquatic organisms. Thus, the residues of antibiotics in the waters of Hong Kong need to be more closely monitored.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.ecoenv.2015.12.002.

References


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References


