Determination of selected antibiotics in the Victoria Harbour and the Pearl River, South China using high-performance liquid chromatography–electrospray ionization tandem mass spectrometry

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Antibiotics were found at high concentration levels in an urban reach of Pearl River in southern China with contrast diurnal variations between the high and low water seasons.

Abstract

Nine selected antibiotics in the Victoria Harbour and the Pearl River at Guangzhou, South China, were analyzed using high-performance liquid chromatography–electrospray ionization tandem mass spectrometry. The results showed that the concentrations of antibiotics were mainly below the limit of quantification (LOQ) in the marine water of Victoria Harbour. However, except for amoxicillin, all of the antibiotics were detected in the Pearl River during high and low water seasons with the median concentrations ranging from 11 to 67 ng/L, and from 66 to 460 ng/L, respectively; and the concentrations in early spring were about 2-15 times higher than that in

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summer with clearer diurnal variations. It was suggested that the concentrations of antibiotics in the high water season were more affected by wastewater production cycles due to quick refreshing rate, while those in the low water season may be more sensitive to the water column dynamics controlled by tidal processes in the river.

Keywords: Antibiotics; Water; High performance liquid chromatography (HPLC); Tandem mass spectrometry; Hong Kong; Pearl River; China

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1. Introduction

In recent years, there has been an increasing interest in the study of occurrence and fate of pharmaceuticals in the aquatic environment because of their potential function toward the spread and maintenance of resistance in bacterial pathogens and post-therapeutic effects. More than 3000 different chemical substances have been used as human medicines, and in aquaculture and farming applications (Ternes et al., 2004), in which antibiotic is one of the most important groups of common pharmaceuticals in our daily lives. It is well known that the principal pathway of antibiotics into the aquatic environment is through wastewater systems following consumption and excretion by humans, and via effluents from landfills, farms and abattoirs (Daughton and Ternes., 1999; Simon et al., 2005). However, conventional wastewater treatment plants (WWTPs) were designed without consideration of antibiotics removal in wastewater. Many previous studies have shown that, while some antibiotics may be eliminated in the WWTPs, some may be hard to be removed in the process, and can therefore reach the aquatic environment. It was reported that more than 16 categories of antibiotics were observed in river water (Hirsch et al., 1998; Golet et al., 2001; Sacher et al., 2001; Ternes et al., 2002; Kolpin et al., 2002; Lindberg et al., 2004; Miao et al., 2004), sludge and soils (Golet et al., 2002; Hamscher et al., 2002; Michael et al., 2003; Golet

et al., 2003).

The annual usage of antibiotics has been estimated between 100,000-200,000 tons globally (Kümmerer., 2003), with more than 25,000 tons in China. In this study, a total nine mostly common antibiotics used in China, selected from five important categories, including quinolones (ofloxacin and norfloxacin), macrolides (roxithromycin and erythromycin), sulfonamides (sulfadiazine，sulfadimidine and sulfamethoxazole) , β-lactam (amoxicillin) and chloramphenicol (chloramphenicol), were analyzed by HPLC-MS-MS technology in water samples collected from the Victoria Harbour, Hong Kong, and the Pearl River at Guangzhou, South China. Located in South China, the Pearl River Delta (PRD) is one of the fast developing and most urbanized regions in China. With a population of more than 40 millions (including Hong Kong), and a wastewater treatment ratio of lower than 50%, it is estimated that pharmaceuticals and personal care products (PPCPs) in the surface water in the Pearl River Delta may deserve a great concern. However, there has been very little research so far on antibiotics in the environment of the region (Richardson et al., 2005).

The aims of the present study were (1) to develop a sensitive method for the determination of selected common antibiotics in various water samples;(2) to determine the selected antibiotics in seawater from the Victoria Harbour of Hong Kong, and the Pearl River water at Guangzhou for an understanding the occurrence of common antibiotics in a subtropical region; (3) to study the diurnal and seasonal variations of antibiotic in the Pearl River water.

2. Materials and methods

2.1. Chemicals and standards

Ofloxacin, norfloxacin, roxithromycin, erythromycin, sulfadiazine, sulfadimidine, sulfamethoxazole, amoxicillin and chloramphenicol were purchased from Sigma-Aldrich Co. $^{13}C_3$-caffeine solution was obtained from Cambridge Isotope Labs (1 mg/ml in methanol, USA）. All the antibiotics were dissolved in methanol and stored in a freezer. Erythromycin-H_2O, major degradation of erythromycin, was obtained by acidification from erythromycin using the method described by Christa et al (2003). Methanol and acetonitrile (HPLC grade) were obtained from Merck (Darmstadt, Germany). Ultra-pure water was prepared with a Milli-Q water purification system (Millipore, Bedford, MA, USA). Unless otherwise indicated, chemicals used were purer than the analytical grade.

2.2. Sampling

Seawater samples were collected in the Victoria Harbour of Hong Kong in December 2004 and February 2005 at five different sites (B1, B2, B3, B4 and B5) (see Fig. 1). Surface and bottom water (1 m above the sediment and water interface) samples were obtained during the sampling cruise and stored in a precleaned distilled water container. The river water samples were collected every two hours over one whole day during the representative high (June, 2005) and low (March, 2005) water seasons at a sampling station located at the urban section of the Pearl River at Guangzhou. All the samples were collected (about 1 m below the surface) using a water grab sampler and stored in pre-cleaned brown glass bottles. All samples (seawater and river water) were kept in the dark at 5 ℃ in a cold storage room before further treatment and analysis.

2.3. Preparation for analysis

The water samples were filtered through 0.45 μm glass fiber filters (Millipore, MA, USA). About one liter of water sample was acidified to pH = 3.0 by adding 3.0 M H_2SO_4, followed by addition of 0.2 g disodium

ethylenediamine tetraacetate (Na_2EDTA). Before the samples were subjected to extraction, 100 ng $^{13}C_3$-caffeine was added to each sample as a surrogate to monitor the recovery. An Oasis HLB cartridge (6 mL, 500 mg, Waters) used for the solid-phase extraction (SPE) was preconditioned sequentially with 6.0 mL of methanol, 6.0 mL of ultra-pure water and 6.0 mL of 10 mmol/L Na_2EDTA buffer (pH 3.0). Thereafter, the samples were passed through the SPE columns at a flow rate of approximately 10 mL/min. The HLB column was then rinsed with 10 mL of ultra-pure water pH 3.0, and dried under nitrogen gas for 1 h. After drying, each cartridge was eluted with 2 mL (× 3) of methanol. The analytes were collected in a 10 mL brown glass vial, volume-reduced under nitrogen purge to about 20 μl, and then dissolved in 40% aqueous methanol to a final volume of 1.0 mL.

For the recovery experiments, one L of filtered groundwater, artificial seawater and river water were fortified separately with 100 ng of target analytes, and 100 ng of surrogate ($^{13}C_3$-caffeine). The solutions were treated in the same procedure as the field samples described above.

2.4. HPLC system

The LC system was an HP 1100 LC (Agilent Technologies, Palo Alto, CA, USA) controlled gradient system. It was equipped with an auto sampler, a pump and a thermostated column oven. An ODS-P (Dikma, USA. 4.6 mm×250 mm i.d., 3.5 μm) chromatograph column was employed and was operated at 35℃. Optimum separation was achieved using gradient elution. Mobile phase consisted of A: acetonitrile; B: water with 0.2% (v/v) formic acid. The gradient was set up as follows: 0–8 min 40% A, 8–10 min a linear gradient to 60% A, 10–25 min 60% A, 25–30 min linear gradient to 40% A, and kept at 40% for 5 min. The injection volume was 20 μl, and the flow-rate was 0.4 mL/min. All the compounds were eluted out of the column within 22 min.

2.5. MS-MS system

Mass spectrometric measurements were performed on a Sciex API 4000^{TM} (Applied Biosystems, Foster City,

CA, USA) equipped with an electrospray ionization source (ESI). The analysis was performed in negative mode for amoxicillin and chloramphenicol, in positive mode for the other compounds. Both positive and negative ions were acquired in multiple reaction monitoring (MRM) mode with a dwell time of 200 ms. For the positive model, the temperature of the heated capillary was 450℃, and the source voltage was set to 5.5 kV. For the negative model, they were set as 400℃ and 4.5 kV, respectively. Nebulizer gas, curtain gas, collision gas, entrance potential and collision cell exit potential were set at the following values: 10, 15, 6 psi, −10 and 13V, respectively.

Declustering potential and collision energy were two key elements which influenced the abundance of the product ion. In order to achieve the highest possible sensitivity of the instrument, declustering potential and collision energy were optimized by direct infusion of the pure analytes to the MS-MS compartment (see Table 1).

3. Results and discussion

3.1. Calibration, recoveries and limits of quantification

Calibration solutions (from 0.1 to 2000 ng/L in six points) were prepared by spiking groundwater with each of the targeted compounds. The reported mean correlation coefficients (r^2) of the calibration curves were higher than 0.998 (Table 2), and the relatively standard deviations for all analytes were lower than 6.5%. To obtain the limits of quantification (LOQ) for the groundwater and seawater samples in the current study, the groundwater and artificial seawater were spiked with different amounts of the analytes (n = 5), and the LOQs were calculated as 3 X the standard deviations of the actual measurements of the groundwater and artificial seawater. For the river water samples, LOQ was difficult to determine because the samples already contained some of the selected analytes and the matrix interference was serious. Therefore, LOQs in the river water were defined as a signal-to-noise (S/N) ratios of 10.

138 The recovery rates of groundwater, artificial seawater and river water are shown in Table 2. The
139 mean recoveries for these spiked antibiotics in groundwater, artificial seawater and river water
140 were higher than 67%, 64% and 61%, respectively. Each compound was quantified under the
141 MRM mode using two highest characteristic precursor ion/product ion transitions. Together with
142 the retention times, the characteristic ions were used to ensure correct peak assignment and peak
143 purity. $^{13}C_3$-caffeine was added as a surrogate standard to all samples prior to the enrichment of
144 the control to avoid possible losses during the analytical procedure. It should be noted that
145 erythromycin was determined in the form of its dehydration product, erythromycin-H_2O.
146 Erythromycin exhibited strong pH sensitivity. Hirsch et al (1998) and Christa et al (2003) showed
147 that erythromycin-H_2O was the predominant form of erythromycin in the aquatic environment. In
148 addition, the degraded product, erythromycin-H_2O, does not exhibit the original antibiotics
149 properties because the orally applied erythromycin has to pass through strongly acidic conditions
150 in the stomach, (Yang et al., 2004).
151
152 *3.2. Occurrence of the selected antibiotics in the Victoria Harbour*
153 Only macrolides and quinolones were detected in a few sampling sites from the Victoria
154 Harbour of Hong Kong. Other antibiotics in all sampling sites were lower than the limit of
155 quantification. Table 3 contains a summary of the results with observed maximum and median
156 concentrations.
157 In Hong Kong, the population is estimated to be 6.9 millions. The wastewater of the city is
158 estimated to be more than 2,000,000 m^3 a day. The Victoria Harbour was one of the main venues
159 for WWTP effluent outfalls in Hong Kong. The absence or low concentrations of antibiotics in the

160 seawater samples from Victoria Harbour implies that the environmental conditions in the Harbour
161 has been recovered significantly with the implementation of Harbour Area Treatment Scheme
162 (HATS). In addition, the exchange of the marine water between the Harbour and the outside sea
163 is highly efficient for the dispersion of many water pollutants. The present results also showed
164 that there were not remarkable differences among selected antibiotics in water between different
165 seasons (Fig. 2). Human-use pharmaceuticals have been shown to reach surface waters primarily
166 via discharge of treated wastewater effluents (Hirsch et al., 1999). In our study, the concentrations
167 of antibiotics in the surface seawater were also obviously higher than that in the bottom water in
168 both seasons.

169 It is also worthy to note that Site B1 and B2 are close to a large fish farming area, Lei Yue Mun
170 (a lot of seafood restaurants there) (Fig. 1). Therefore, the elevated concentrations of some
171 antibiotics at these sampling sites may be mainly associated with the intensive fish farming
172 activities in the Harbour, rather than the sewage outfalls.

173

174 *3.3. Occurrence of the selected antibiotics in the Pearl River*

175 The results from the two sampling campaigns (March and June, 2005) of the Pearl River at
176 Guangzhou are presented in Table 4. Except for amoxicillin, all the selected antibiotics were
177 detected in both the high and low water seasons. The median concentrations of antibiotics in the
178 high and low seasons ranged from 11 to 67 ng/L, and from 66 to 460 ng/L, respectively.

179 Guangzhou has a population of more than 10 millions at the end of 2004, and it is estimated that
180 more than 1.7 million tons of domestic wastewater were produced per day in the city. The Pearl
181 River is the sole receiving water body for the wastewater, treated or untreated. The wastewater

182 treatment ratio for domestic sewage was only 61.7% in Guangzhou at the end of 2004, leaving
183 about 0.65 million ton of wastewater per day discharged directly to the Pearl River without any
184 treatment. More new WWTPs are under construction in order to achieve a wastewater treatment
185 ratio of 70% by the end of 2005, and 100% by 2007.

186 Our data showed that the urban section of the Pearl River in Guangzhou was still seriously
187 contaminated by some common antibiotic. It would be of great interest to monitoring these
188 pollutants in river water with the continuous implementation of new WWTPs in the city in the
189 next few years.

190 Significant differences were observed for all the antibiotics under study between the two
191 sampling times. Considerably higher concentrations were found during the sampling campaign in
192 March 2005 (low water season) than June 2005 (high water season with storm water and flooding).
193 Storm water and flooding may either input additional antibiotics from agricultural land and fish
194 ponds to the river, or dilute the concentrations of these pollutants in river water. In the Pearl River
195 Delta region, June is one of the most typical months in the rainy seasons. In June 2005, especially
196 from the 20^{th} to the 29^{th}, due to the concurrence of continuous rainfall and astronomical tides, the
197 water level of the urban section of the Pearl River at Guangzhou reached its 100-year record (13 m,
198 63,200 m^3/s), which was 1.5 m higher than the security water level in the city. In contrast, the
199 water level in March 2005 was only 2 - 3 m, with a water flow of 2500-3000 m^3/s. The lower
200 concentrations of antibiotics in the June 2005 suggested a dominant process of dilution in the Pearl
201 River in high water season, and further indicated that domestic sewages, irrespective of rainfall,
202 may be the principal source for antibiotics in the river water.

203 Ofloxacin and norfloxacin were only detected at two sampling times during the 24 h

204 sampling cycle in the high water season. However, they were found at all the sampling times in
205 the low water season, with the concentrations ranging from 53 to 108 ng/L, and from 117 to 251
206 ng/L, respectively. Quinolone concentrations in the low water season were much high than those
207 in the river water of the Glatt Valley Watershed, Switzerland (Golet et al., 2002), and were
208 comparable and within the range of those previously reported in wastewater in Switzerland and
209 Sweden (Golet et al., 2003; Lindberg et al., 2005), which might reflect the relatively high use rate
210 of quinolones in China.

211 Macrolides were detected in both the high and low water seasons. Erythromycin is readily
212 dehydrated by loss of one water molecule, and its dehydration product erythromycin-H_2O has
213 been detected predominantly in the environment (Gobel et al, 2005). Macrolides were detected in
214 the high water season in similar concentration ranges of the Poudre River in USA (Yang et al.,
215 2004), with concentrations ranging from 5 to 105 ng/L for roxithromycin, and from 13 to 423 ng/L
216 for erythromycin-H_2O. In Germany and Canada, macrolides have been detected in all WWTP
217 effluents with high concentrations of roxithromycin and erythromycin-H_2O. Roxithromycin, was
218 detected in the Pearl River with a median concentration of 66 ng/L in the low water season.
219 Erythromycin-H_2O concentrations (median 460 ng/L, maximum 636 ng/L) in the low water season
220 were comparable and within the range of normal WWTPs in Canada (Miao et al., 2004).

221 In the high water season, the concentrations (median) of sulfadiazine, sulfadimidine and
222 sulfamethoxazole were 38, 67 and 37 ng/L, respectively. The maximum concentrations of
223 sulfadiazine, sulfadimidine in the low water season were 336 and 323 ng/L, respectively. The
224 sulfamethoxazole concentrations (111-193 ng/L) in the low water season were much higher than
225 that in the Höje River, Sweden (David et al., 2005) and the surface water in German (Hartig et al.,

226 1999). The extensive usage of sulfonamides in various applications contributes to the frequent
227 detection of them in the environment. In addition, sulfonamides have a high potential to resist
228 degradation, and are thus hydrophilic enough to be transported into the aquatic environment
229 (Zuccato et al., 2001).

230 Amoxicillin, the most frequently prescribed antibiotic in China, was not found in both high
231 and low water seasons. The potential explanation could be that the lactam class of antibiotics
232 readily undergoes hydrolysis shortly after excretion due to the chemically unstable lactam ring
233 (Gáspár et al., 2002), and this degradation processes may be enhanced in the subtropical water
234 environment, such as Hong Kong and Guangzhou.

235 Chloramphenicol has widely been used to treat animals since the 1950s. Besides, the external
236 use of chloramphenicol in aquaculture and human application has been rather extensive in China.
237 Chloramphenicol was detected in both the high and low water seasons in the Pearl River, with
238 concentrations ranging from 11 to 266 ng/L, and from 54 to 187 ng/L, respectively.

239

240 *3.4. Diurnal variations of antibiotics concentrations in the Pearl River*

241 The discharge of domestic wastewater in an urban area may display a diurnal pattern as a
242 sequence of daily life cycles of the residents. The Guangzhou section of the Pearl River is also
243 tidal, with two flood tides and ebb tides every day. In the present study, diurnal variations of the
244 antibiotics concentrations in the Pearl River were monitored by sampling the water every two
245 hours through a full day (24 h cycle). Fig. 3 and 4 show the diurnal variations of antibiotics
246 concentrations in the high water season and the low water season, respectively. Also depicted in
247 the figures are the relative water levels recorded every two hours at the water sampling station.

248 Macrolides, sulfonamides and chloramphenicol were detected most frequently, while
249 quinolones were only detected in a few sampling times in the high water season. However, all the
250 compounds were detected in the river water samples during the low water season. The antibiotics
251 concentrations in the high water season showed a strong diurnal pattern. It is interesting to note
252 that much higher concentrations of antibiotics in the river water were observed, despite of the high
253 water level, in 20:00 hrs, 22:00-01:00 hrs and 08:00 hrs, which corresponded with the major
254 domestic sewage production times (via untreated outfalls).

255 On the contrary, the antibiotics concentrations in the low water season displayed only weak
256 diurnal variations. However, it was found that the concentrations tended to increase with the rising
257 of the water level as a result of the flood tide twice a day. When a flood tide comes from the lower
258 reach, the relatively dense saline water would probably enhance the resuspension of bed sediments
259 and/or the mixing processes in the water column. Previous studies indicated that the amount of
260 antibiotics sorbed to suspended solids and bed sediments can not be neglected (Hektoen et al.,
261 1995; Halling-Sorensen et al., 1998; Löffler et al., 2003; Beausse, 2004; Lalumera et al., 2004).
262 Therefore, it is suggested that, in the low water season, the enhanced resuspension of bed
263 sediments and the mixing processes in the water column by tidal intrusion may be responsible for
264 the relatively high concentrations of antibiotics in the Pearl River at Guangzhou.

265 The interesting contrast diurnal variations of antibiotics concentrations in the river water
266 during the high and low water seasons may deserve more detailed study. As in the high water
267 season, the flow rate in the river was much higher than that in the low water season, due to the
268 rainfalls and surface runoffs from the watershed; this will by no means enable a quick
269 refresh/replacement of water in the urban river section. In the meantime the dynamic processes

such as mixing and resuspension in the river water body may be less affected by the tidal processes. Therefore, the antibiotics concentrations in the water may be more depended on the *in situ* discharge of wastewater and daily sewage production cycles. In the low water season, the refreshing rate of the river water is lower, leading to a relatively longer residence time of antibiotics in the urban river section, as may mask the variations caused by daily sewage production cycles, while the tidal processes may strongly affect the dynamics of the water body in the absence of significant contribution from storm/rain waters.

4. Conclusions

The method developed for the determination of antibiotics was successfully applied to the analysis of seawater and river water samples. The use of the MRM model can improve the precision and sensitivity of the analysis. In the Victoria Harbour of Hong Kong, the concentrations of antibiotics were mainly below the limit of quantification (LOQ). However, the selected antibiotics were found in the Pear River during the high and low water seasons at 10s to 100s ng/L levels. The concentrations of antibiotics in the low water season were much higher than those in the high water season, but with a less significantly diurnal variation. It is suggested that the antibiotics concentrations in the high water season was more controlled by daily sewage discharge patterns due to quick refreshing rates, while those in the low water season may be more sensitive to the water column dynamics as enhanced by tidal processes.

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375 **Table 1**

376 **Optimized MS/MS parameters for the target antibiotics**

Compound	Precursor ion	Product ion	Declustering potential (*V*)	Collision energy (*V*)
Ofloxacin	362 $[M+H]^+$	318 $[M-CO_2+H]^+$	130	24
Norfloxacin	320 $[M+H]^+$	302 $[M-H_2O+H]^+$	120	25
Roxithromycin	837.1 $[M+H]^+$	158.2[desosamine $+H]^+$	120	54
Erythromycin-H_2O	716.1 $[M+H]^+$	558.3[M+H-desosamine-$H_2O]^+$	120	25
Sulfadiazine	250.9 $[M+H]^+$	92.2	70	40
Sulfadimidine	279.1 $[M+H]^+$	186.1[M-aminophonyl]$^+$	75	25
Sulfamethoxazole	253.9 $[M+H]^+$	156.1[Sulfonamidyl cation]$^+$	68	26
Amoxicillin	363.8 $[M-H]^-$	205.6	-80	-25
Chloramphenicol	321 $[M-H]^-$	151.6	-100	-25
$^{13}C_3$-caffeine	198.1 $[M+H]^+$	140.1	80	25

377

Table 2

Linearity of calibration, recoveries and limits of quantification of the analytes in groundwater (GW), artificial seawater (ASW) and river water (RW).

Compound	R^2	Recovery ± SD (%)			LOQ[a] (ng/L)		
		GW	ASW	RW	GW	ASW	RW
Ofloxacin	0.998	88±10	84±12	81±12	1.5	2.6	10
Norfloxacin	0.998	86±4	77±7	80±5	2.2	3.2	10
Roxithromycin	0.998	85±12	78±9	78±11	0.4	2.0	5
Erythromycin-H_2O	0.998	91±11	72±6	81±9	0.4	2.0	5
Sulfadiazine	0.999	91±4	88±4	86±4	0.2	0.5	1
Sulfadimidine	0.999	90±5	90±3	79±5	0.2	0.5	1
Sulfamethoxazole	0.999	88±7	85±10	84±9	1.0	0.8	1
Amoxicillin	0.998	67±15	64±11	61±10	3.2	5.0	20
Chloramphenicol	0.998	74±8	78±18	80±12	2.4	4.1	5
$^{13}C_3$-caffeine	0.999	93±6	88±10	83±8	0.3	0.4	1

[a] Limits of quantification.

383 **Table 3**

384 **Summary results for the selected antibiotics in seawater from the Victoria Harbour, Hong Kong**

Compound	No. of samples[a]	Concentrations of the antibiotics in seawater in December 2004 (ng/L)			Concentrations of the antibiotics in seawater in February 2005 (ng/L)		
		No.＞LOQ[b]	Median	Maximum	No.＞LOQ	Median	Maximum
Ofloxacin	10	5	5.2	8.1	6	10	16.4
Norfloxacin	10	4	9.4	28.1	4	12.3	20.1
Roxithromycin	10	3	6.1	21.1	5	5.1	30.6
Erythromycin-H_2O	10	2	3.3	5.2	3	3.4	4.2
Sulfadiazine	10	0	nd[c]	nd	0	nd	nd
Sulfadimidine	10	0	nd	nd	0	nd	nd
Sulfamethoxazole	10	0	nd	nd	0	nd	nd
Amoxicillin	10	0	nd	nd	0	nd	nd
Chloramphenicol	10	0	nd	nd	0	nd	nd

385 [a] Samples from the five sampling sites (including surface and bottom water)

386 [b] Limits of quantification.

387 [c] Not detected.

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401 **Table 4**

402 **Summary results for the selected antibiotics in the Pearl River water samples during the high and low water**

403 **seasons**

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Compound	No. of samples[a]	Concentrations of the antibiotics in river water (high water season, ng/L)			Concentrations of the antibiotics in river water (low water season, ng/L)		
		No.＞LOQ[b]	Median	Maximum	No.＞LOQ	Median	Maximum
Ofloxacin	12	2	11	16	12	77	108
Norfloxacin	12	2	12	13	12	150	251
Roxithromycin	12	11	16	105	12	66	169
Erythromycin-H_2O	12	12	30	423	12	460	636
Sulfadiazine	12	12	38	141	12	209	336
Sulfadimidine	12	12	67	179	12	184	323
Sulfamethoxazole	12	12	37	165	12	134	193
Amoxicillin	12	0	nd[c]	nd	0	nd	nd
Chloramphenicol	12	12	41	266	12	127	187

405 [a] Samples each campaign from the Pearl River during every two hours over one whole day.

406 [b] Limits of quantification.

407 [c] Not detected.

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Figures Captions

Fig. 1. Sketch map showing the sampling sites

Fig. 2. Concentrations of the selected antibiotics at each sampling location of the Victoria Harbour in December 2004 and February 2005.

Fig. 3. Mean concentrations (n = 3) of the selected antibiotics in each sampling time during one day in the low water season of the Pearl River.

Fig. 4. Mean concentrations (n = 3) of the selected antibiotics in each sampling time during one day in the high water season of the Pearl River.

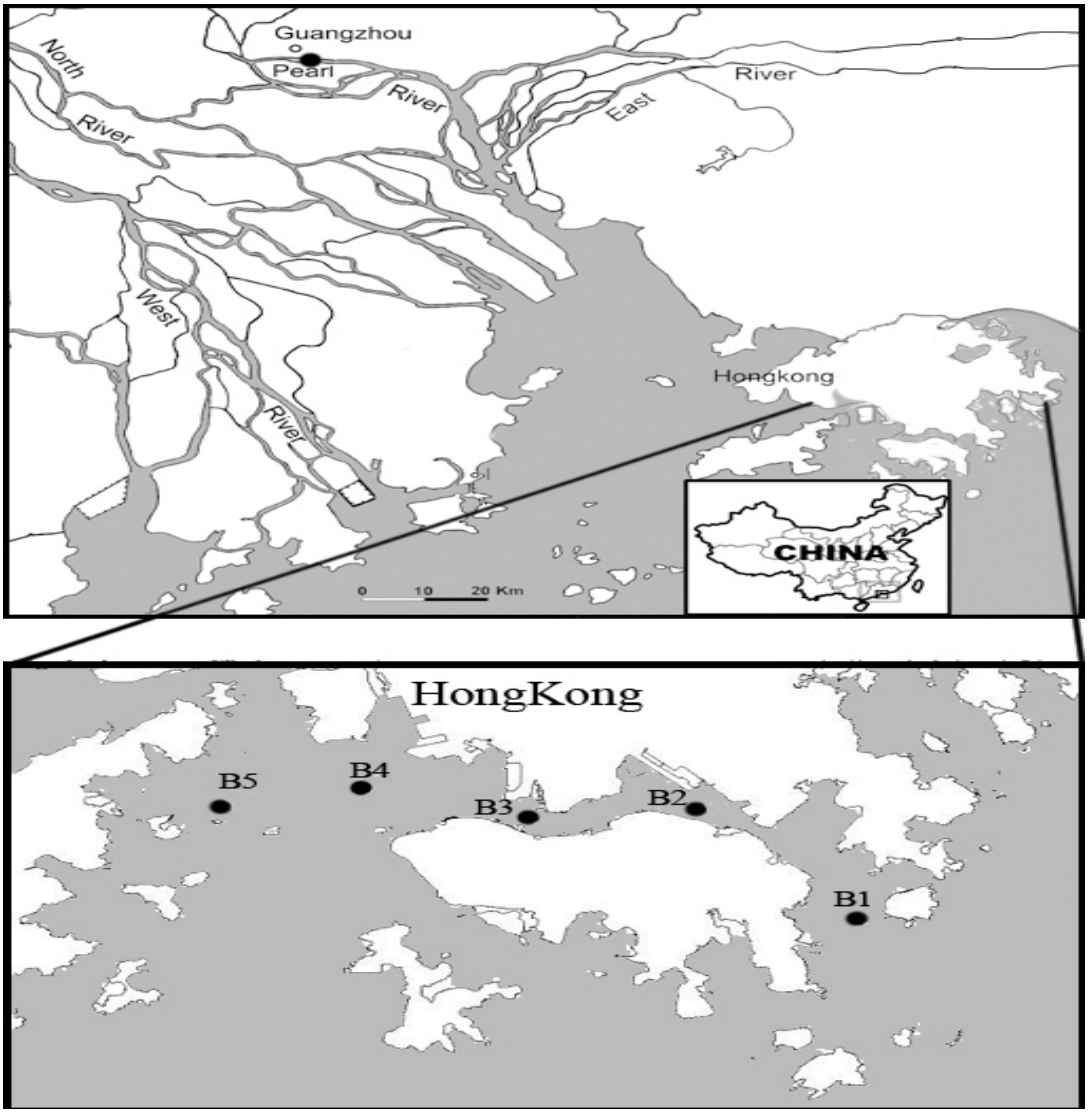


Fig. 1. Sketch map showing the sampling sites

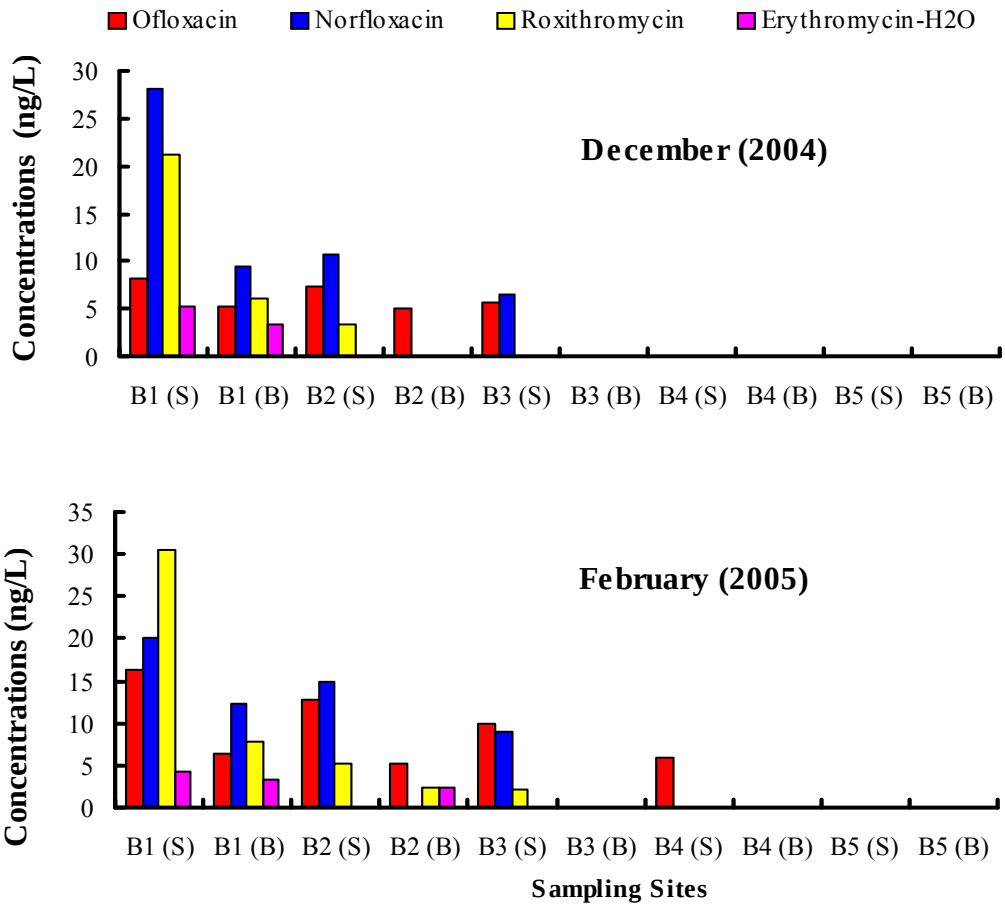


Fig. 2. Concentrations of the selected antibiotics in each sampling location of the Victoria Harbour in December 2004 and February 2005.

(S) - surface water

(B) - bottom water

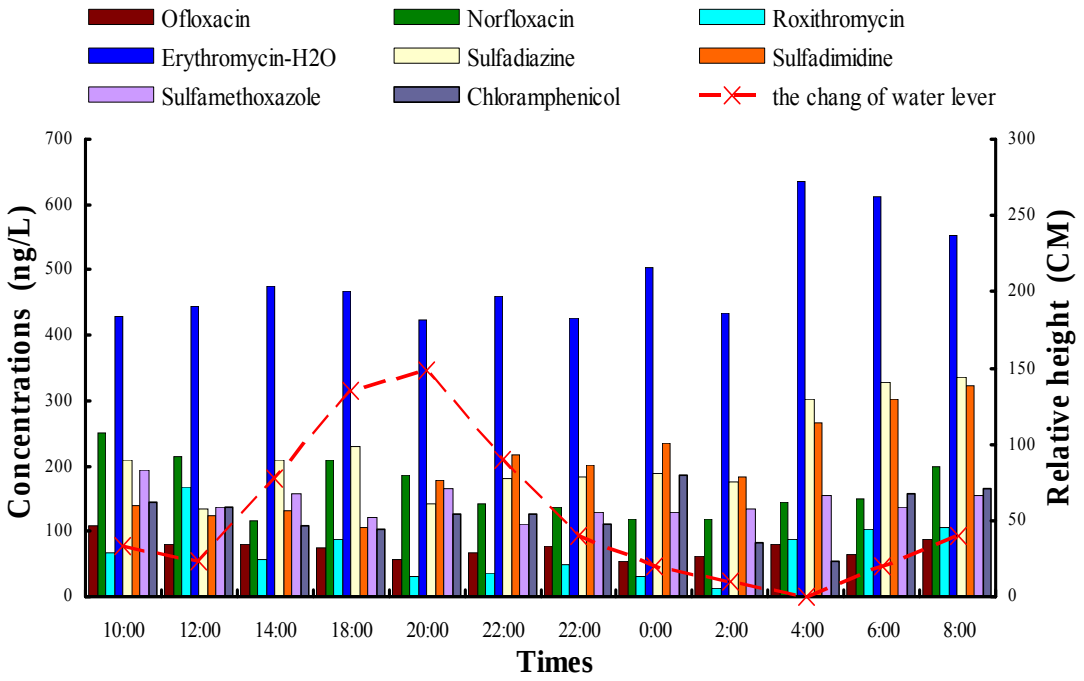
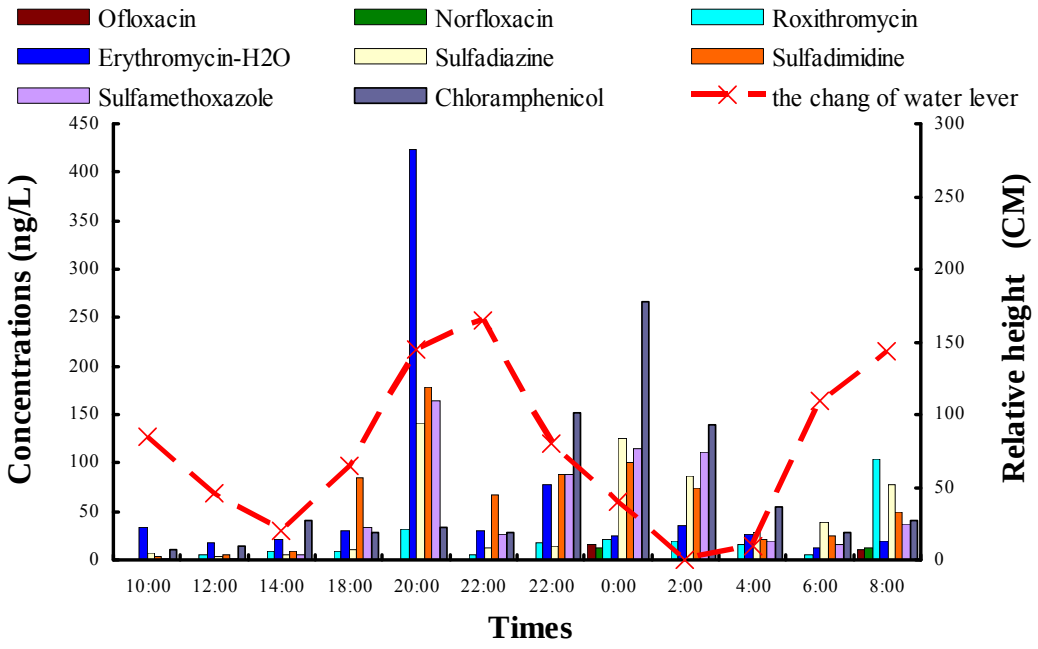


Fig. 3. Mean concentrations (n=3) of the selected antibiotics in each sampling time during one day in the low water season of the Pearl River.



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462 **Fig. 4. Mean concentrations (n=3) of the selected antibiotics in each sampling time during one day in the**
463 **high water season of the Pearl River.**

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