Provision of Services for Laboratory Analysis of Antibiotics in Ambient River and Marine Water Samples

Final Report

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1. Introduction

Antibiotics have received environmental concern as emerging contaminants since the late 1990s because of their ubiquitous occurrence, ecotoxicity and ability to induce antibiotic resistance in organisms (Carvalho and Santos, 2016). Sources of antibiotics into the environment mainly include: wastewater discharge, direct application in aquaculture and excretion from the livestock after treatment of antibiotics and illegal disposal (Carvalho and Santos, 2016, Yang et al., 2018). Through transportation via surface runoffs and/or other land-based systems, residues of antibiotics could find their way into the marine environment, posing potential ecological risks. Recently, risk quotients of three antibiotic mixtures to certain algal species in European surface waters were reported as high as 385, implying a high likelihood of adverse impacts on algal communities which often play an important role in the primary production and nutrient cycling of aquatic systems (Guo et al., 2016). To date, several antibiotics have been on the watch list under the Water Framework Directive of the European Commission, revealing environmental significance of these drugs (Loos et al., 2018).

Several studies have previously reported the occurrence of several antibiotics in different environmental matrices, including wastewater, surface water and sediment in Hong Kong, which observed no high risks posed to the aquatic organisms in most cases (Deng et al., 2016, Deng et al., 2018, Gulkowska et al., 2007, Gulkowska et al., 2008, Leung et al., 2012, Minh et al., 2009, Selvam et al., 2017, Wilkinson et al., 2022).

Levels of antibiotics were also found in tap water and food from the market (meat, eggs and milk) and children's urine previously, yet no significant human health risks were identified (Leung et al., 2013, Li et al., 2017). The above results were also in line with the conclusion drawn by the World Health Organization (WHO) that it was extremely unlikely to cause appreciable health risk through the exposure of pharmaceuticals via drinking water (WHO, 2012).

To respond to the WHO's announcement on the issue of emerging antibiotic resistance genes (ARGs) in the environment, the Government of the Hong Kong Special Administrative Region launched the Hong Kong Strategy and Action Plan on Antimicrobial Resistance (2017-2022) and measures have been taken to regulate improper use of antibiotics (HKCHP, 2017). In turn, the environmental profiles of antibiotics might have changed with the possibly shifting prescription pattern in Hong Kong. Consequently, more up-to-date information about the occurrence of antibiotics in Hong Kong wastewater and surface waters is needed.

In connection to the above, the Environmental Protection Department have contracted the City University of Hong Kong to conduct a baseline survey study from 2020 to 2021, which covered three major sewage treatment works, nine river stations and 11 marine stations included (locations and description of the sampling sites are shown in **Figure 1** and **Table 1**). This project aims to:

1) investigate the occurrence of 26 commonly prescribed antibiotics in treated wastewater effluent, river water and seawater in two wet and two dry seasons from across Hong Kong from 2020 to 2021;

2) determine the levels of antibiotics in Hong Kong waters, their possible sources and seasonal pattern;
3) compare the results of this study with other previous relevant studies and evaluate the potential ecological risks to the aquatic organisms and of causing antimicrobial resistance;
4) acquire baseline data for further investigations as necessary.

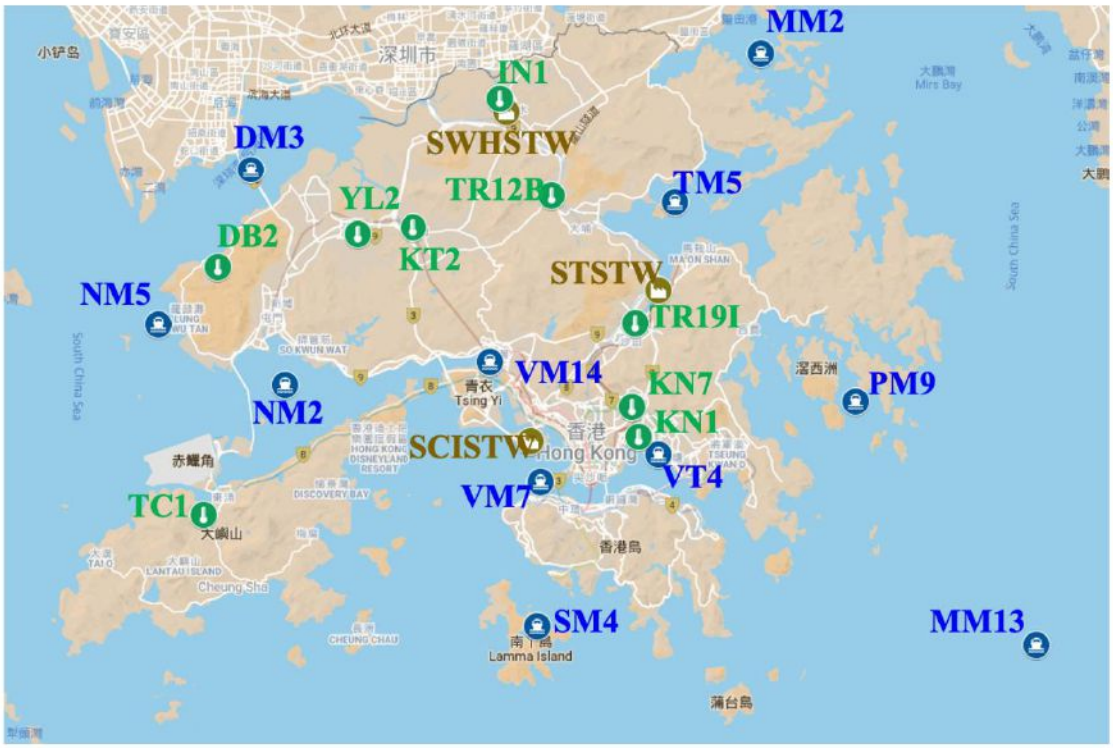

Figure 1. Locations of the sampling stations (Blue dots: Marine stations; Green dots: River stations; Brown dots: Sewage treatment works)

Table 1. Codes and description of the sampling sites

Station code	Description
Sewage treatment works	
SCISTW	Stonecutters Island Sewage Treatment Works, chemically enhanced primary treatment (CEPT) plant, receiving wastewater from both shores of Victoria Harbour with a designed flow of 2,450,000 m^3/d and serving population of 5.7 million[a].
STSTW	Shatin Sewage Treatment Works, the largest traditional activated sludge treatment plant in Hong Kong receiving wastewater of Shatin New Town with a designed flow of 340,000 m^3/d and serving population of 0.6 million. The treated effluents of STSTW combined with those from Tai Po Sewage Treatment Works are being discharged into Victoria Harbour via Kai Tak River under the Tolo Harbour Effluent Export Scheme (THEES).
SWHSTW	Shek Wu Hui Sewage Treatment Works, a traditional activated sludge treatment plant, receiving both domestic and slaughterhouse wastewater located in the North District and part of Yuen Long District with a designed flow of 93,000 m^3/d and serving population of 0.3 million.

River stations

TC1	Tung Chung River, a river located in Lantau Island. It is chosen as the control station for inland water courses because of its low level of *E. coli* as monitored by EPD
IN1	River Indus, a major river in North District receiving local discharges from North District as well as SWHSTW
KN1	The downstream station of Kai Tak River receiving treated effluents discharged from STSTW and Tai Po STW via the THEES as well as surface runoffs along Kai Tak River
KN7	The upstream station of Kai Tak River, just below the effluent discharge point from THEES
KT2	Kam Tin River receiving surface runoffs, domestic effluents as well as possible discharges from local livestock farms
DB2	Tai Shui Hang Stream, a station near the West New Territories Landfill site
TR12B	Lam Tsuen River receiving discharges and surface runoffs from Lam Tsuen and surrounding areas
TR19I	Shing Mun River receiving discharges and surface runoffs from Shatin New Town areas
YL2	Yuen Long Creek receiving surface runoffs, domestic effluents as well as possible discharges from local livestock farms

Marine stations

MM13	The marine control station located at the southeast water of Hong Kong
MM2	Located in Mirs Bay near marine park and fish culture zones
TM5	Located in Inner Tolo Harbour near the fish culture zone at Yim Tin Tsai
PM9	Located in outer Port Shelter near some mariculture sites
SM4	Located in southern water near Sok Ku Wan (SKW) fish culture zone and the outfall of SKWSTW
VM14	Located in Tsuen Wan Bay and northwest of SCISTW effluent outfall. Also receiving surface discharges from 3 major local culverts
VM7	Located west of Victoria Harbor, possibly influenced by discharges from SCISTW outfall

VT4	Kwun Tong Typhoon Shelter, receiving discharges from Kai Tak River and the overflow from Kowloon Bay Sewage Interception Station via the Kai Tak Approach Channel
DM3	Located near oyster culture areas in inner Deep Bay and influenced by Shenzhen River
NM2	Located in northwestern waters, near the outfall of Pillar Point STW
NM5	Located in northwestern waters near the Urmston Road effluent outfall. A site most likely influenced by discharges of Pear River especially in the wet season

a: https://www.dsd.gov.hk/SC/HTML/141.html

2. Antibiotics pollution state in wastewater effluent, river water and seawater in 2020–2021

2.1. Average levels of the target antibiotics (as total antibiotics (Σantibiotics))

Overall, the average levels (in medium bound[1], same hereafter) of ∑antibiotics in STW's effluent, river water and seawater sampled at 4 occasions during two wet and two dry seasons from September 2020 to November 2021 in Hong Kong ranged from 6.37 ng/L (PM9) to 3220 ng/L (SCISTW) in the wet season and 5.10 ng/L (DB2) to 2310 ng/L (SCISTW) in the dry season (**Figure 2**). Average number of target antibiotics detected in wastewater effluent, river water and seawater were 22, 15 and 11 out of the 26 target antibiotics (Table 2).

2.1.1 Sewage Treatment Works

In treated effluent from the three major STWs, the highest levels of ∑antibiotics were found in the largest SCISTW (with a designed flow of 2,450,000 m^3/d and serving population of 5.7 million) at 3220 ng/L and 2310 ng/L, whereas the lowest ∑antibiotics level was observed in the smallest SWHSTW (with a designed flow of 93,000 m^3/d and serving population of 0.3 million) at 263 ng/L and 378 ng/L in wet and dry seasons, respectively. The levels of ∑antibiotics in STSTW (with a designed flow of 340,000 m^3/d and serving population of 0.6 million) were 1320 ng/L and 1730 ng/L in wet and dry seasons.

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[1] 'Medium-bound' means concentrations of individual antibiotics below LOQ were assigned a value of half of the limit of quantification (½LOQ) for calculating the∑antibiotics.

Levels of ∑antibiotics found in these three STWs increased with the designed capacities of the STWs. One of the possible reasons may be related to the densities of hospitals and clinics located in their corresponding catchments. Around 80% antibiotics supply went to the private doctors and Hospital Authority which managed public hospitals and institutions in Hong Kong in 2020 (HKCHP, 2022). Although the distribution of private clinics in Hong Kong was unavailable, it was clearly shown in **Figure 3** provided by the Hong Kong Hospital Authority that more public hospitals and institutions were located in both shores of Victoria Harbour where SCISTW served, followed by the catchments STSTW and SWHSTW served (HKHA, 2022). The other reason could be attributed to the difference in removal efficiencies of antibiotics by the investigated STWs. In two previous studies, consistently higher removal of antibiotics (i.e., erythromycin-H_2O, tetracycline, norfloxacin, sulfamethoxazole, trimethoprim, cefalexin and chloramphenicol) were observed in STSTW than that in SCISTW (Gulkowska et al., 2008, Leung et al., 2012). All ß-lactams, sulfamethoxazole and chloramphenicol were reported to be rapidly dissipated by the traditional activated sludge treatment deployed in STSTW (Leung et al., 2012). In a lab-scale study, Suarez et al. (2009) also reported nil or poor removal of antibiotics including sulfamethoxazole, trimethoprim, roxithromycin and erythromycin after coagulation-flocculation treatment by using ferric chloride (also used by SCISTW) and aluminum sulfate as coagulants. Despite that studies on the removal of pharmaceuticals by CEPT were scarce, the lower removal performance consistently observed could be possibly attributed to the absence of biodegradation process during CEPT (Wu et al., 2020).

Consistent lower levels of antibiotics were observed in the wet season than those in the dry season in STSTW and SWHSTW, both of which employed traditional activated sludge treatment. This observation could be possibly related to the higher temperature in the wet season which favored the proliferation of microorganisms. The optimum temperature in activated sludge system typically ranged from 30 to 38 ºC (Eggen and Vogelsang, 2015).

2.1.2 Inland Water Courses

In river water, Kam Tin River (KT2), Kai Tak River (KN1 and KN7), Yuen Long Creek (YL2), and River Indus (IN1) were found to have higher levels of antibiotics. Among these locations, the highest level of ∑antibiotics was detected at KT2 at 2980 ng/L in the wet season. This level was comparable to those found in SCISTWs. Nevertheless, markedly lower ∑antibiotics level (771 ng/L) was observed at KT2 in the dry season.

Similar to STSTW, the levels of ∑antibiotics were found to be comparable in wet and dry seasons at Kai Tak River, where 1290 ng/L (wet) and 1340 ng/L (dry) were observed at the upstream site (KN7) and 1030 ng/L (wet) and 960 ng/L (dry) at the downstream site (KN1). It should be noted that KN7 should be more strongly influenced by the discharges from THEES while the downstream KN1 station would also be subjected to the influence of stormwater discharges and surface runoffs from southeastern Kowloon area along Kai Tak River particularly during the wet season.

Unlike Kai Tak River, higher levels of ∑antibiotics were identified at IN1 (444 ng/L) and YL2 (544 ng/L) in the dry season than those (IN1: 147 ng/L; YL2: 207 ng/L)

in the wet season possibly due to the dilution effect of stormwater discharges and surface runoffs during the wet season.

Varied levels of ∑antibiotics within the same season of two sampling years were observed at Lam Tsuen River (TR12B) and the main channel of Shing Mun River (TR19I) with average levels of 21.3 ng/L and 47.1 ng/L in the wet season and 19.2 ng/L and 53.6 ng/L in the dry season. The varying levels of antibiotics detected in the same seasons possibly implied the existence of discontinuous or local pollution sources at these two sampling sites.

Levels of ∑antibiotics at Tai Shui Hang Stream in Deep Bay (DB2), at 14.1 ng/L and 5.10 ng/L in the wet and dry season, were the lowest among all the river stations, even lower than the control station (TC1).

Regarding to the number of antibiotics detected, subject to the influence of effluent discharge from STSTW and Tai Po STW, 23 antibiotics were found at Kai Tak River in the Kowloon area, the highest among the river stations. A relatively higher numbers of antibiotics (i.e., 22 at KT2, 20 at IN1 and 15 at YL2) were also detected in the Northwestern New Territories (except DB2), where treated and/or untreated sewage as well as livestock farms might be the sources (Section 2.2). Less number of antibiotics were found in the Eastern New Territories (8 at TR12B and 10 at TR19I) and that in DB2 was even less than the control station (**Table 2**).

2.1.3 Marine Waters

In seawater samples, the highest levels of ∑antibiotics were detected at Kwun Tong Typhoon Shelter (VT4) at 660 ng/L and 339 ng/L in wet and dry season respectively.

The levels at VT4 were one or two orders of magnitude higher than those of other marine stations because it received the polluted riverine runoff from Kai Tak River as well as overflows of polluted stormwater runoff from Kowloon Bay Sewage Interception Station to Jordan Valley Box Culvert compounded by the static hydraulic condition around this sampling site located inside the typhoon shelter. Among other marine stations, the levels of ∑antibiotics at stations located at the northwestern waters (DM3, NM5 and NM2), near western Victoria Harbour (VM14 and VM7) and southern waters (SM4), ranging from 17.5 ng/L (NM2) to 43.1 ng/L (VM7) in the wet season and 11.8 ng/L (SM4) to 32.4 ng/L (VM7) in the dry season. The levels of ∑antibiotics found in Mirs Bay (MM2), Tolo Harbour (TM5), Port Shelter (PM9) located in eastern Hong Kong ranged from 6.37 ng/L (PM9) to 12.8 ng/L (TM5) in the wet season and 8.05 ng/L (TM5) to 9.24 ng/L (PM9) in the dry season, which were quite comparable to those found at the control station (MM13) and lower than the other marine stations located at the northwestern waters, near western Victoria Harbour and southern waters. Similar trend could also be inferred from the number of antibiotics detected at these marine stations from different water zones in Hong Kong (**Table 2**).

Overall, no consistent seasonal pattern was observed in wastewater effluent, river water and seawater samples collected in Hong Kong. As for the geographic orientation of the sampling stations, for river stations, higher levels of antibiotics were found in the rivers located in the Northwestern New Territories than those in the Eastern New Territories, possibly because livestock farms and unsewered village houses were more abundant in the Northwestern New Territories. Both located in the Eastern New

Territories, levels of antibiotics were higher in Shing Mun River (Shatin catchment) than those in Lam Tsuen River (Tai Po catchment), which could be attributed to a higher population density in Shatin than in Tai Po. For marine stations, higher levels of antibiotics were observed in the Northwestern Waters and Victoria Harbour than those in the Southern Waters and the Eastern Waters. This observation could be possibly ascribed to more freshwater runoff originated from local farms and the influence of Pearl River runoff in the Northwestern Waters and higher population density in the Victoria Harbour catchment, which was positively correlated to the number of hospitals located (**Figure 3**).

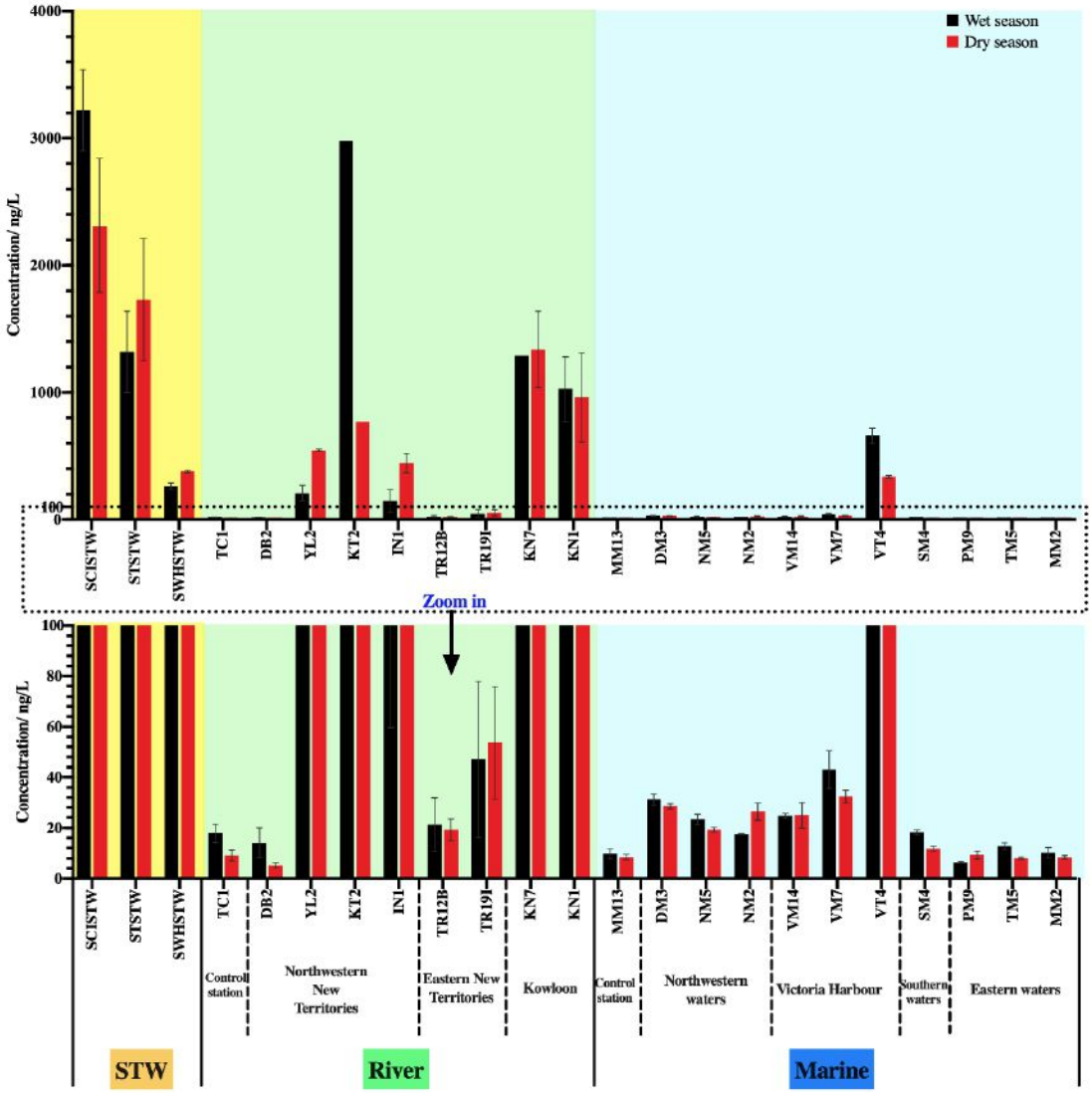

Figure 2. Concentrations (ng/L) of ∑antibiotics detected in wet season (September 2020 and August 2021) and dry season (December 2020 and November 2021) in wastewater effluent, river water and seawater in Hong Kong. Error bar represents the level ranges detected in two sampling campaigns within one wet/dry season.

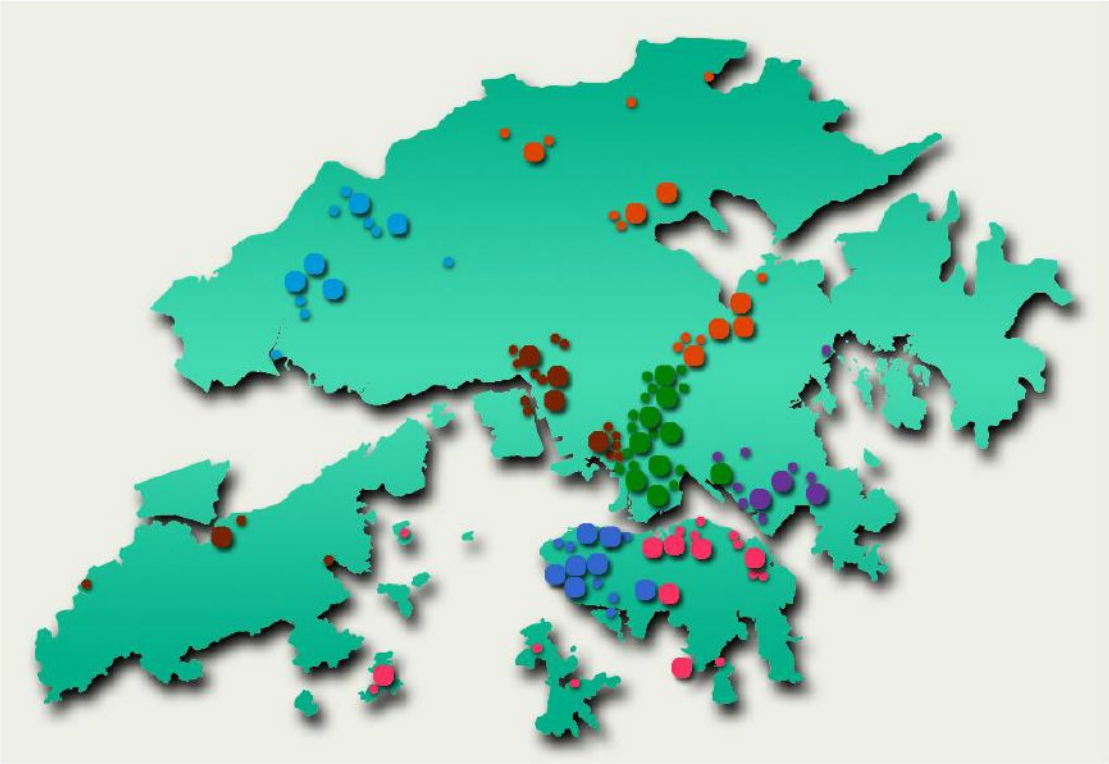

Figure 3. The distribution of clusters, hospitals and institutions managed by Hong Kong Hospital Authority (different colors of dots represent clusters in different areas of Hong Kong). Adopted from https://www.ha.org.hk/visitor/ha_visitor_index.asp?Content_ID=10036&Lang=ENG&Dimension=100&Parent_ID=10004&Ver=HTML.

Table 2. Number of antibiotics detected out from the 26 target antibiotics in different sampling stations in the four sampling campaigns

Matrices	Areas	Stations	Wet season	Dry season	All
Wastewater effluent		SCI	25	23	24
		ST	24	22	23
		SWH	21	20	21
River water	Control station	TC1	10	4	7
	Northwestern New Territories	DB2	8	4	6
		YL2	14	17	15
		KT2	22	21	22
		IN1	20	20	20
	Eastern New Territories	TR12B	11	6	8
		TR19I	10	9	10
	Kowloon	KN1	25	21	23
		KN7	25	21	23
Seawater	Control station	MM13	8	6	7
	Northwestern Waters	DM3	13	11	12
		NM5	13	11	12
		NM2	10	12	11
	Victoria Harbour	VM14	14	12	13
		VM7	16	12	14
		VT4	22	18	20
	Southern Waters	SM4	12	8	10
	Eastern Waters	PM9	8	8	8
		TM5	9	6	7
		MM2	10	7	9
Average		Wastewater effluent	23	22	22
		River water	16	14	15
		Seawater	12	10	11

2.2. Composition profile of the target antibiotics

The composition profiles of antibiotics in this study was generally comparable between wet and dry seasons in most of the sampling sites, indicating that their sources of antibiotics were consistent and continuous over the 2-year sampling period (**Figure 4**). Therefore, the composition values were reported as average of four sampling campaigns unless specified.

2.2.1 Sewage Treatment Works

In general, fluoroquinolones (ofloxacin: 19.3%–25.6%, ciprofloxacin: 14.3%–19.0%) and macrolides (azithromycin: 8.42%–10.7%, clarithromycin: 4.86%–12.4%), which are mainly for human use in Hong Kong (Table 1), predominated in the treated effluent samples of STSTW, SWHSTW and SCISTW. The composition profiles of antibiotics were similar between the two biological STWs, STSTW and SWHSTW. However, the proportions of penicillins (i.e., piperacillin) (7.67%) and sulfonamides (12.4%) were higher in effluent samples of SWHSTW. In addition, sulfamethazine (1.08%), which is only for veterinary use in Hong Kong, was consistently detected in four sampling campaigns at SWHSTW. It was detected at an average of 3.31 ng/L. Sulfamethoxazole, which is a drug that only prescribed in combination with its potentiator trimethoprim for human use in Hong Kong but widely applied for veterinary use in mainland China (Zhang et al., 2015) accounted for 5.42% of the composition in the effluent samples collected in SWHSTW, and was markedly higher compared with the other two STWs (SCISTW: 0.636%; STSTW: 0.619%). These observations implied that SWHSTW received wastewater of animal origin, i.e., slaughterhouse and/or the

nearby livestock farms. On the other hand, apparent difference in the proportion of cefalexin was found in the effluent samples of SCISTW. Cefalexin accounted for 12.3% of the composition profile in SCISTW effluent, which could be attributed to inefficient removal performance of cefalexin by chemically enhanced primary treatment deployed at SCISTW (Leung et al., 2012).

2.2.2 Inland Water Courses

Kai Tak River

In river water samples, the composition profiles of ∑antibiotics at KN7 and KN1 were predominated by fluoroquinolones (ofloxacin and ciprofloxacin) and macrolides (azithromycin and clarithromycin), which was similar to that of STSTW, indicating that Kai Tak River was mainly subject to the influence of THEES. Slightly higher levels of ∑antibiotics found in the upstream (KN7) than those in the downstream (KN1, receiving stormwater) with similar composition profile also implied relatively minor contribution of antibiotics from stormwater/run-off compared with THEES.

River Indus

At the downstream of the effluent outfall of SWHSTW, treated effluent from SWHSTW should be one of the main sources contributing to the antibiotic present at IN1. However, the composition profile of antibiotics at IN1, which was dominated by penicillins, macrolides, sulfonamides and fluoroquinolones, were not quite similar to that of SWHSTW. The proportion of sulfadiazine (26.6%), trimethoprim (13.3%), sulfamethazine (2.34%) in IN1 were apparently higher than those in SWHSTW effluent (sulfadiazine: 5.91%; trimethoprim: 5.07%; sulfamethazine: 1.08%). Besides, cefalexin,

extensively prescribed for human use (100% in Hong Kong and 95.2% in mainland China) was detected at IN1 at 11.4 ng/L, accounted for 4.02% of the antibiotic composition. It accounted for only 0.121% and 0.886% in the effluent samples of SWHSTW and STSTW, respectively. These secondary sewage treatment works employed activated sludge which can effectively remove cephalosporins, including cefalexin (Leung et al., 2012). The higher proportion of cefalexin implied untreated sewage (i.e., unsewered village houses, expedient connections in the old districts) could be a possible source of antibiotics in IN1.

Yuen Long Creek

Sulfadiazine dominated and accounted for 57.0% of the composition profile of YL2. Sulfamethazine was found at 15.8 ng/L (6.03%), while ofloxacin accounted for 11.7% of the composition profile. These three antibiotics were extensively prescribed for human use in Hong Kong but mainly for animal use in mainland China (sulfadiazine: 81.1%; sulfamethazine: 89.9%; ofloxacin: 75.0%) (Zhang et al., 2015). Cefalexin accounted for 1.66% of the composition profile, which was higher than those in STSTW and SWHSTW but lower than that of IN1. The possible sources include runoff from unsewered village houses, expedient connections in the old districts and discharges from the upstream livestock farms.

Kam Tin River

The composition profiles of KT2 were quite different between wet and dry seasons. Sulfadiazine (70.0%) and trimethoprim (18.8%) were predominated in the wet season, whereas sulfadiazine (20.4%), doxycycline (32.2%) and lincomycin (14.9%) were

dominated in the dry season. Doxycycline and lincomycin were both prescribed for human and animal use in both Hong Kong and mainland China (Zhang et al., 2015). Both of them were more frequently prescribed for human use in Hong Kong, while extensively used in animal husbandry and aquaculture (lincomycin: 87.2%; doxycycline: 94.8%), especially swine (lincomycin: 55.5%; doxycycline: 60.4%) in mainland China (Zhang et al., 2015). Sulfamethazine, was found to be 44.6 ng/L at KT2. This level was the highest among all the sampling sites across Hong Kong. Furthermore, KT2 was the only sampling station at which tylosin, only for veterinary usage, was detected. Considering the livestock farms in the nearby area, the observation implied wastewater of animal origin in addition to human origin are likely sources of antibiotics detected in KT2. The distinct seasonal pattern in composition profile observed at KT2 suggested inconsistent and irregular sources existed, which needed further investigation.

Lam Tsuen River

Sulfadiazine and cefalexin dominated and accounted for 27.9% and 21.3% of the composition profile of TR12B. The composition of cefalexin was greatly higher than those of other rivers, implying untreated sewage as a possible source.

Comparison with the control station (Tung Chung River)

In sum, most of the river stations were unique in the composition profiles, compared with the control station TC1 (Tung Chung River), suggesting these stations were influenced by antibiotics with difference sources.

2.2.3 Marine Waters

Kwun Tong Typhoon Shelter

In seawater samples, the composition profile at VT4 was similar to those of STSTW and Kai Tak River, indicating the treated effluent discharge under the THEES was the main source of antibiotics. Higher proportion of ß-lactams was found at VT4 (16.4%), compared with that at KN1 (downstream of Kai Tak River, 9.17%), possibly indicating additional source of stormwater/surface runoffs.

Victoria Harbour

Likely subject to the influence of SCISTW effluent discharges, a relatively large proportion of cefalexin was found at VM7 (west side of Victoria Harbour, 12.7%) and VM14 (Tsuen Wan Bay, 15.3%). Runoff from the three culverts possibly carrying untreated sewage may also be a source to VM14. In addition, sulfadiazine was predominated in the composition profiles of seawater samples collected at VM7 (37.1%) and VM14 (23.8%). In Hong Kong, sulfadiazine was prescribed mainly for human use (topical use only) and occasionally for veterinary use (**Table A1**). Considering no livestock farms near these two marine stations, antibiotics pollution at Victoria Harbour were most likely originated from human uses.

Southern Waters

SM4 is located near the effluent discharge of Sok Kwu Wan STW in the Southern WCZ. The composition profile of antibiotics in seawater samples collected at SM4 was similar to those at VM7 and VM14. No evidence suggested that the SM4 was affected by the nearby fish culture zone.

Northwestern Waters and Deep Bay

DM3, NM2 and NM5 are located at the northwestern waters of Hong Kong. The

major antibiotics detected in seawater samples collected at these sites include sulfadiazine, trimethoprim, erythromycin-H_2O, lincomycin and chlortetracycline. Their antibiotics composition patterns were distinct from those collected in the Southern Waters and Eastern Waters of Hong Kong. Sources of antibiotics pollution in the northwestern waters may include influence of the Pearl River discharges, local runoffs and treated effluent discharged from northwestern New Territory as well as livestock farms. Discharges from Shenzhen River can also be a source of antibiotics contributed to Deep Bay.

Comparison with the control station (MM13)

Compared with the composition profile of the control station MM13, more diverse antibiotics composition patterns were found at stations in the western side because of multiple antibiotics sources, while those found in the eastern side were similar.

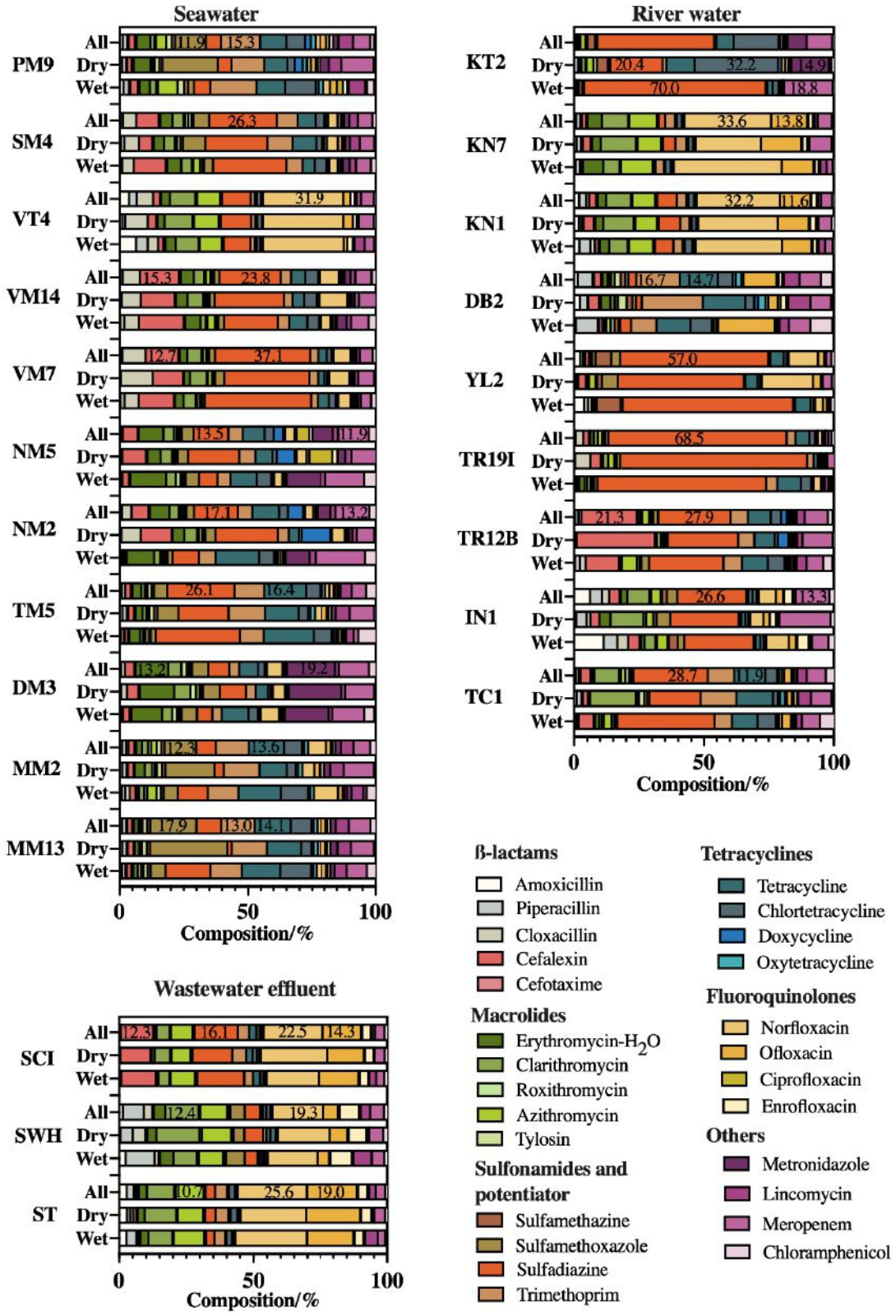

Figure 4. Seasonal composition profiles of antibiotics in wastewater effluent, river water and seawater in Hong Kong. For KT2 (Kam Tin River), only one dry season and one wet season were analyzed. For KN7 (Kai Tak River), only one wet season was analyzed.

2.3. Level comparison with previous publication

Table 3 compared the average levels of antibiotics detected in the present survey with previous publications at the same sampling stations[2]. Overall, the concentrations of most antibiotics detected in the present study were found to be much lower than those reported previously (Deng et al., 2016; Minh et al., 2009; Wilkinson et al., 2022), especially macrolides and fluoroquinolones (i.e., erythromycin-H_2O, roxithromycin, norfloxacin and ofloxacin). For example, the levels of clarithromycin (132 ng/L) and ciprofloxacin (188 ng/L) at Kai Tak River (i.e. KN7) in the present study were much lower than those (324 ng/L and 306 ng/L, respectively) recently reported by Wilkinson et al. (2022) which sampled river water in 2018. The level of sulfamethoxazole at KN7 in the present study was much lower (by more than 15 times) compared with those sampled by Wilkinson *et al* in 2018 (see Wilkinson et al., 2022). The observed relatively low levels of antibiotics at Kai Tak River suggested that the stricter regulation on the prescription of antibiotics in Hong Kong (HKCHP, 2022) has reduced the consumption of antibiotics in recent years. According to statistics, the proportion of antibiotics supplied to community pharmacies decreased from 18.5% in 2016 to 7.5% in 2020, indicating harder accessibility of antibiotics in Hong Kong (HKCHP, 2022). Levels of oxytetracycline and norfloxacin were < 0.151 ng/L and < 0.280 ng/L at the

[2] Antibiotics that were not detected or lower than detection limits in other studies were not included for comparison because "not detected" of a target analyte was governed by the detection limits of different methods and it was not fair to compare "not detected" values having higher detection limits (other studies) with detected values having lower detection limits (present study) of the same analyte among different studies.

west side of Victoria Harbour (VM7) in the present study, whereas high levels of oxytetracycline (132 ng/L) and norfloxacin (1500) were recorded in study sampled in 2008 (Minh et al., 2009), which could be also attributed to the integrative sewage collection from areas in both side of Victoria Harbour to SCISTW under the Harbour Area Treatment Scheme (HATS). However, except sulfamethazine, higher levels of other antibiotics were found at Kam Tin River (i.e. KT2) in the present study than those of Deng's study published in 2016. For example, sulfadiazine was found to be 1120 ng/L in the present study while 1.3 ng/L in Deng's study at KT2. Further investigation at Kam Tin River may be needed due to the relatively high levels and inconsistent seasonal patterns of antibiotics found at KT2.

Table 3. Comparison on the concentrations (ng/L) of the detected antibiotics in the present study with previous publication.

Site	AMX	CLX	CTX	CTM	ETM-H_2O	RTM	SMZ	SMX	SDZ	TC	OTC	DC	NFX	OFX	CFX	TMP	MTZ	CAP	Reference
SCISTW	NA	1800	NA	NA	510	NA	NA	NA	NA	510	NA	NA	320	NA	NA	230	NA	NA	(Gulkowska *et al.*, 2008)
	NA	1290	NA	NA	455	120	NA	110	NA	152	71	NA	364	980	NA	91	NA	234	(Minh *et al.*, 2009)
	NA	326	NA	NA	29.2	3.91	NA	16.1	NA	107	16.5	NA	13.9	631	NA	91.1	NA	17.7	Present study
STSTW	NA	330	34	NA	600	NA	NA	NA	NA	NA	NA	NA	100	NA	NA	120	NA	NA	(Gulkowska *et al.*, 2008)
	NA	179	NA	NA	358	NA	NA	37	NA	NA	31	NA	77	708	NA	68	NA	NA	(Leung *et al.*, 2012)
	NA	12.0	3.21	NA	49.9	NA	NA	7.77	NA	NA	7.83	NA	10.7	416	NA	51.5	NA	NA	Present study
SWHSTW	NA	NA	NA	NA	NA	NA	NA	0.7	NA	NA	NA	13.1	NA	135.9	NA	NA	NA	NA	(Deng *et al.*, 2016)
	NA	NA	NA	NA	NA	NA	NA	16.3	NA	NA	NA	5.14	NA	62.8	NA	NA	NA	NA	Present study
IN1	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	26.4	NA	4.3	NA	NA	NA	NA	(Deng *et al.*, 2016)
	NA	NA	NA	NA	NA	NA	5.83	17.3	NA	NA	NA	NA	NA	2.9	NA	NA	NA	NA	(Deng *et al.*, 2018)
	NA	NA	NA	NA	NA	NA	5.01	12.4	NA	NA	NA	3.75	NA	21.1	NA	NA	NA	NA	Present study
KT2	NA	NA	NA	NA	NA	NA	288.9	1.5	1.3	NA	NA	17.9	NA	0.4	NA	NA	NA	NA	(Deng *et al.*, 2016)
	NA	NA	NA	NA	NA	2.78	29.49	29.9	NA	NA	NA	4.36	NA	0.38	NA	NA	NA	NA	(Deng *et al.*, 2018)
	NA	NA	NA	NA	NA	0.331	44.6	10.5	1120	NA	NA	158	NA	14.5	NA	NA	NA	NA	Present study
KN7	NA	NA	NA	324	NA	NA	NA	89.4	NA	NA	NA	NA	NA	NA	306	35.4	9.46	NA	(Wilkinson *et al.*, 2022)
	NA	NA	NA	132	NA	NA	NA	5.68	NA	NA	NA	NA	NA	NA	188	70.9	27.0	NA	Present study
TR19I	NA	NA	NA	NA	NA	NA	0.7	NA	NA	NA	NA	NA	NA	0.2	NA	NA	NA	NA	(Deng *et al.*, 2016)
	NA	NA	NA	NA	NA	NA	0.131	NA	NA	NA	NA	NA	NA	0.614	NA	NA	NA	NA	Present study
VT4	8	88	NA	NA	212	11.5	NA	16	NA	NA	NA	NA	NA	125	NA	53	NA	NA	(Minh *et al.*, 2009)
	25.0	12.3	NA	NA	19.2	1.02	NA	2.75	NA	NA	NA	NA	NA	156	NA	21.9	NA	NA	Present study
VM7	1.9	8.6	NA	NA	25	NA	NA	NA	NA	NA	132	NA	1500	9.9	NA	NA	NA	NA	(Minh *et al.*, 2009)
	0.252	4.69	NA	NA	1.19	NA	NA	NA	NA	NA	< 0.151	NA	< 0.280	2.40	NA	NA	NA	NA	Present study

Note: Abbreviations (AMX: amoxicillin; CLX: cefalexin; CTX: cefotaxime; CTM: clarithromycin; RTM-H_2O: erythromycin-H_2O; SMZ: sulfamethazine; SMX: sulfamethoxazole; SDZ: sulfadiazine; TC: tetracycline; OTC: oxytetracycline; DC: doxycycline; NFX: norfloxacin; OFX: ofloxacin; CFX: ciprofloxacin; TMP: trimethoprim; MTZ: metronidazole; CAP: chloramphenicol); NA represents not available due to any of the following reason: not analyzed or not detected or lower than detection limit.

2.4. Risk assessment

Generally, no significant difference (Wilcoxon signed-rank test, $p < 0.05$) was observed on the levels of individual antibiotics between wet and dry seasons. Therefore, concentrations of the target antibiotics detected across the four sampling campaigns were pooled for risk assessment. Risk quotients (RQs) were calculated to evaluate the environmental risks of individual antibiotics posed on the aquatic organisms (RQ-ENV) and of causing antimicrobial resistance (RQ-AMR). The median levels of individual antibiotics detected from nine river stations and 11 marine stations across the four sampling campaigns were adopted as measured environmental concentrations to evaluate RQs under the normal-case scenario. To be conservative, the highest levels of individual antibiotics were also adopted to evaluate the worst-case scenario. Method of risk assessment was described in the Appendix.

In the normal-case scenario, no RQ-ENV and RQ-ARM of individual antibiotics was found to be larger than unity, indicating that there were insignificant risks to the aquatic organisms and of causing antimicrobial resistance posed by the presence of antibiotics in the Hong Kong waters (**Figure 5a**).

In the worst-case scenario, the RQ-ENVs of two macrolides, clarithromycin and azithromycin were larger than unity, indicating that they may pose risks to the aquatic organisms in surface waters of Hong Kong. On the other hand, RQ-AMR of ciprofloxacin was larger than unity, which suggested that ciprofloxacin may cause antimicrobial resistance in the Hong Kong waters (**Figure 5b**).

Probabilistic risk assessment was further conducted to evaluate the probability of causing risks in surface waters of Hong Kong by these three antibiotics. Results showed that the probabilities of clarithromycin, azithromycin and ciprofloxacin to pose ecological risks were insignificant, which were at 4.3%, 18.5% and 7.9%, respectively (**Figure 5c**). All these three antibiotics were found to have RQ > 1 at Kai Tak River. RQ values of azithromycin were also larger than unity at Kam Tin River and Kwun Tung Typhoon Shelter. Notably, clarithromycin, azithromycin and ciprofloxacin, accounting for 5.58%, 5.42% and 2.91%, respectively, of the whole antimicrobials supply in 2020, were among the top 10 most commonly supplied antimicrobials in 2016–2020 in Hong Kong (HKCHP, 2022).

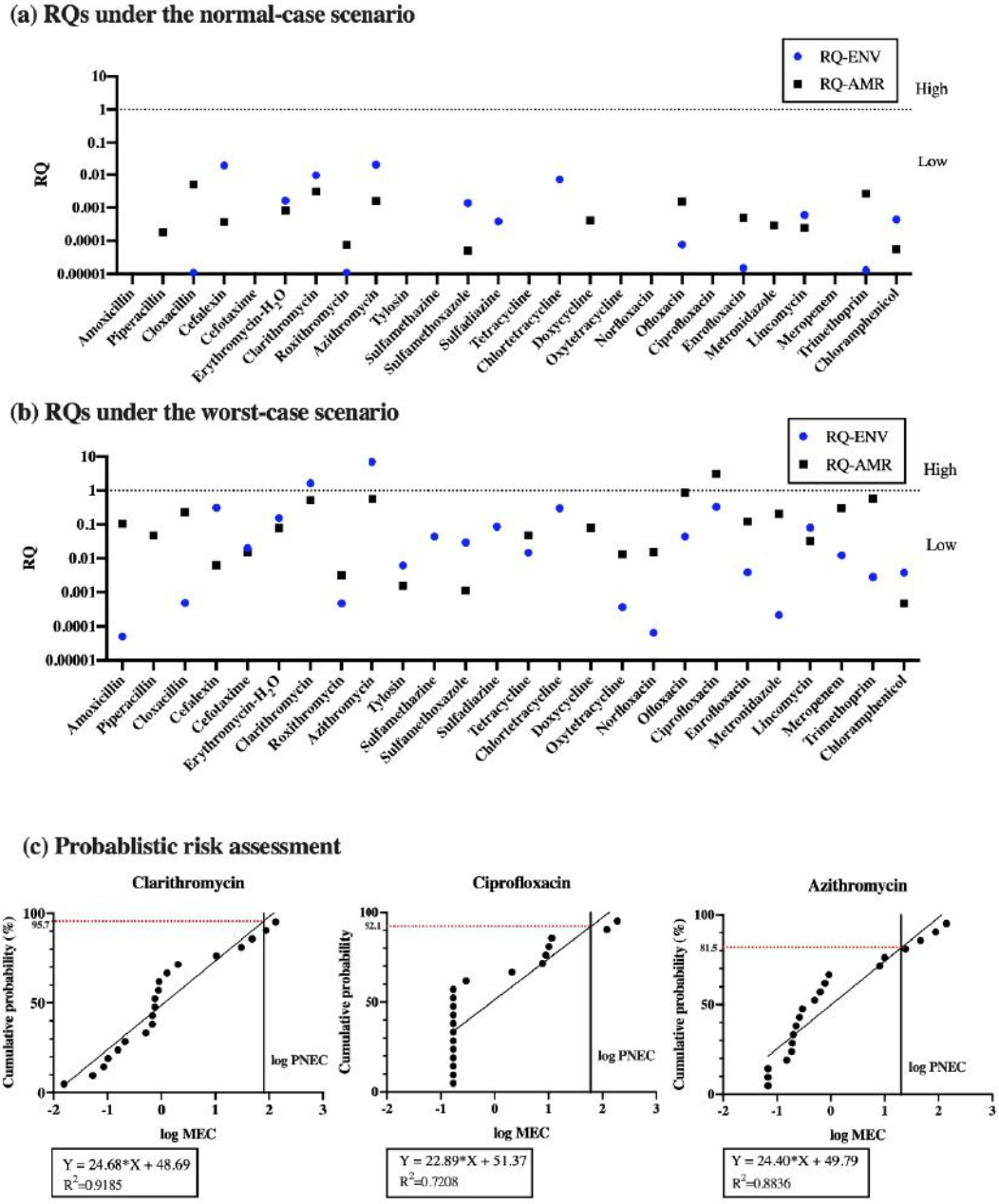

Figure 5. Risk quotients of antibiotics posed to the aquatic environment (RQ-ENV) and of causing antimicrobial resistance (RQ-AMR) under (a) normal-case scenario and (b) worst-case scenario; Dots are not shown in the figure for those values <0.00001; (c) Probabilistic risk assessment for clarithromycin, azithromycin and ciprofloxacin (n = 20). Linear regression equations with R^2 values are shown. Values at the red dotted lines represent the probabilities that the antibiotics do not pose risks.

3. Summary and recommendations

In general, levels of ∑antibiotics in wastewater effluent, river water and seawater in Hong Kong ranged from 5.10 ng/L to 2980 ng/L with no distinct pattern between dry and wet seasons. Higher levels of antibiotics were found at Kam Tin River, River Indus and Yuen Long Creek with various sources, including treated or untreated effluent discharges, runoffs from expedient connections and livestock farms as well as stormwater and/or surface runoffs. Higher levels of antibiotics were also found in Kai Tak River and Kwun Tong Typhoon Shelter because Kwun Tong Typhoon Shelter receives discharges from THEES via Kai Tak River and Kwun Tong typhoon shelter also receives overflows of polluted stormwater runoff from Kowloon Bay Sewage Interception Station to Jordan Valley Box Culvert compounded by the static hydraulic condition inside the typhoon shelter.

Composition-wise, sulfadiazine and ofloxacin predominated in most of the sampling stations. The composition profiles of antibiotics at Kam Tin River, Lam Tsuen River and Shing Mun River varied across seasons, implying discontinuous and possible local pollution sources. With regard to Kai Tak River and Kwun Tong Typhoon Shelter, main source of antibiotics was identified as treated sewage discharge from STSTW and Tai Po STW under THEES since they had similar composition profiles with that of STSTW. Regarding marine water, further investigations with more sampling stations will be needed to better confirm their sources. More sampling effort should be spent on Northwestern Waters and Victoria Harbour to better identify the overall risks posed by antibiotics in these water areas.

Overall, concentrations of most antibiotics in this study were found to be lower in the present study than those reported previously including the most recent report by Wilkinson et al. in 2022. The findings of this study showed that the median levels of these antibiotics in our environmental waters were generally lower than or comparable to those levels reported in other densely populated cities such as London in the UK, Las Vegas in the USA and Seoul in the South Korea (Wilkinson et al., 2022). The risk assessments conducted in this study suggested that the median levels of the antibiotics in this survey were lower than the internationally accepted "Predicted No Effect Concentration (PNEC)", indicating insignificant ecological risks of these antibiotics found in our aquatic environment at least at this time. To further minimize the environmental risks of antibiotics in Hong Kong, it is recommended that EPD should continue to keep in view of relevant international research studies and the progress in the development of environment criteria for residual antibiotics, such as whether international authorities like the World Health Organization will set up any environmental safety level for antibiotics.

Both EPD and the Drainage Services Department (DSD) should also review any new or more effective treatment technologies that can be adopted by the STWs, with the aim to further reduce pharmaceuticals residues including antibiotics in the treated effluent. Specifically, it was suggested that increasing the solid retention time (SRT) during activated sludge treatment favored the growth of a more heterogenic microbial community and thus increased the probability of biodegradation of antibiotics (e.g., trimethoprim) (Eggen and Vogelsang, 2015). We also suggest to use chemical and

microbial source tracking method to identify the sources of antibiotics (human or animal origins). For example, caffeine was used as an indicator of sewage pollution from human origin (Peeler et al., 2006). Adenoviruses and JC polyomaviruses were used as human markers while porcine adenoviruses and bovine polyomaviruses were used as porcine and bovine markers in surface waters of the Europe (Rusinol et al., 2014).

Appendix

Materials and methods

Clinical use of target antibiotics

Information on the clinical use of the target antibiotics that have been discussed above are listed in **Table A1** for reference.

Table A1. Clinical uses of main antibiotics found in seawater, river water and wastewater effluent samples

Antibiotic	Class	Clinical use
Piperacillin	Penicillins	**Human use only in Hong Kong**: mostly in combination with tazobactam (an ß-lactamase inhibitor) to treat pelvic inflammatory disease, intra-abdominal infection, pneumonia, cellulitis and sepsis. It is given by injection into a vein.
Cefalexin	Cephalosporins	**Human use only in Hong Kong**: treatment of certain bacterial infections including middle ear, bone and joint, skin and urinary tract; treatment of certain types of pneumonia, strep throat and prevention of bacterial endocarditis.
Erythromycin-H_2O	Macrolides	**Human use only in Hong Kong**: treatment of bacterial infections including respiratory tract infections, skin infections, chlamydia infections, pelvic inflammatory disease and syphilis; prevention of Group B streptococcal infection and eye ointment in the newborn.
Azithromycin	Macrolides	**Human use only in Hong Kong**: treatment of prophylaxis of surgical infections, respiratory tract infections, skin and soft tissue infections; susceptible Gram-negative infections, susceptible Gram-positive infections; prophylaxis of neonatal conjunctivitis; superficial ocular infections; acne vulgaris
Clarithromycin	Macrolides	**Human use only in Hong Kong**: treatment of bacterial infections including pneumonia, *Heliobacter pylori*, strep throat

		as an alternative of penicillin, cat scratch disease, other infections due to bartonellosis and cryptosporidiosis; as second line agent in Lyme disease and toxoplasmosis; upper and lower respiratory tract infections; encephalitis; primary prevention for Mycobacterium Avium Complex (MAC) bacteremia infection.
Sulfadiazine	Sulfonamides	**Human use**: treatment of toxoplasmosis (with pyrimethamine), otitis media, prevention of rheumatic fever, chancroid and infections by chlamydia and *Haemophilus influenzae*. **Veterinary use**: (in combination with trimethoprim in Hong Kong) treatment of bladder and prostate infections, Nocardia infections or parasitic infections in livestock and pets; enteric redmouth disease, furunculosis, hemorrhagic septicemia, vibriosis and ulcerative diseases in aquaculture.
Sulfamethazine	Sulfonamides	**Veterinary use only in Hong Kong**: in combination with tylosin as swine feed additive to maintain weight gains and feed efficiency in the presence of atrophic rhinitis, to lower the incidence and severity of *Bordetella Bronchiseptica rhinitis*, to prevent swine dysentery associated with *Brachyspira hyodysenteriae* and to control swine pneumonias caused by bacterial pathogens.
Sulfamethoxazole	Sulfonamides	**Human use only in Hong Kong**: in combination with trimethoprim to treat urinary tract infections, methicillin-resistant *Staphylococcus aureus* skin infections, travelers' diarrhea, respiratory tract infections and cholera; treatment and prevention of pneumocystis pneumonia and toxoplasmosis in people with HIV/AIDS and other causes of immunosuppression.

Trimethoprim	Potentiator of sulfonamides	**Human use**: individually or mostly in combination with sulfamethoxazole (refer to "Sulfamethoxazole" in the above). **Veterinary use**: in combination with sulfadiazine (refer to "Sulfadiazine" in the above) or sulfadimethoxine to treat pneumonitis, bronchopneumonia, bronchitis, enteritis, gastroenteritis, peritonitis, metritis, mastitis, colibacillosis, septicemias, equine adenitis, phlegmon, abscesses, wounds, pre and post- surgical treatments of cattle, horses, pigs and sheep.
Chlortetracycline	Tetracyclines	**Human use only in Hong Kong**: as eye ointment to treat eye infections against chlamydiae, rickettsiae, spirochetes, many aerobic and anaerobic gram-negative and gram-positive pathogenic bacteria and some protozoa.
Ofloxacin	Fluoroquinolones	**Human use only in Hong Kong**: treatment of acute bacterial exacerbations of chronic obstructive pulmonary disease, community-acquired pneumonia, uncomplicated skin and skin structure infections, nongonococcal urethritis and cervicitis, epididymitis, acute pelvic inflammatory disease, uncomplicated cystitis, complicated urinary tract infections and prostatitis.
Ciprofloxacin	Fluoroquinolones	**Human use**: treatment of infections including bones and joints, endocarditis, gastroenteritis, malignant otitis, externa, respiratory tract infections, cellulitis, urinary tract infections, prostatitis, anthrax and chancroid. **Veterinary use**: not used in Hong Kong, but could be main metabolite of enrofloxacin, a vet-use only antibiotic in Hong Kong.
Lincomycin	Lincosamides	**Human use**: similar in antibacterial spectrum and mechanism of action to

	macrolides; also effective against actinomycetes and some species of *Mycoplasma* and *Plasmodium*; rarely used because of its adverse effects and toxicity; reserved for patients allergic to penicillins or where bacteria have developed resistance. **Veterinary use**: in combination with spectinomycin to treat respiratory and enteric diseases, foot infections, mastitis, clostridiosis and mycoplasmosis for cattle, sheep and goats, to treat bacterial and mycoplasma pneumonitis, hemorrhagic dysentery, septic arthritis, bacterial enteritis and hemophile pneumonitis for swine, to treat osteomyelitis, septic arthritis and exposed fractures for dogs and cats.

Note: data of human use and veterinary use were obtained from the Drug Office, Department of Health, Hong Kong Special Administrative Region (https://www.drugoffice.gov.hk/eps/do/en/consumer/search_drug_database.html).

Standards and reagents

All native standards were obtained from Toronto Research Chemicals (Toronto, Ontario, Canada), except tetracycline, oxytetracycline and ciprofloxacin which were purchased from Sigma Aldrich (St. Louis, MO, USA). Internal standards (IS) and surrogate were purchased from Toronto Research Chemicals (Toronto, Ontario, Canada), including norfloxacin-d_5, ofloxacin-d_8, $^{13}C_6$-sulfamethazine, ^{13}C-erythromycin-d_3, azithromycin-d_3, roxithromycin-d_7, $^{13}C_4$-cloxacillin sodium salt, doxycycline-d_3 hyclate, chloramphenicol-d_5 and $^{13}C_3$-caffeine. All the standards had purities of >95%. The standards of cloxacillin, piperacillin, tetracycline, chlortetracycline, doxycycline, oxytetracycline and meropenem were prepared in methanol/Milli-Q (*v/v* = 1:1); amoxicillin, cefalexin and cefotaxime were prepared in Milli-Q water; clarithromycin, roxithromycin, azithromycin, tylosin, sulfamethazine, sulfamethoxazole, sulfadiazine, ofloxacin, chloramphenicol, metronidazole, lincomycin and trimethoprim were prepared in methanol; norfloxacin, ciprofloxacin and enrofloxacin were prepared in methanol with 0.02% formic acid (FA). Erythromycin-H_2O were prepared by dissolving erythromycin powder in methanol with 0.02% FA and shaking at 250 rpm for 4 hours; all the surrogate and IS were prepared in methanol, except norfloxacin-d_5 which was prepared in methanol with 0.02% FA.

Methanol of UPLC grade and FA (Suprapur®, 98-100%) were purchased from Merck KGaA (Darmstadt, Germany). Ultrapure water was obtained using an EMD Millipore Milli-Q Gradient water system (Billerica, MA, USA).

Ethylenediaminetetraacetic acid disodium salt (Na_2EDTA) (ACS reagent grade, ≥ 99.0%) was purchased from Sigma-Aldrich (St. Louis, MO, USA).

Extraction, clean-up and instrumental analysis

Samples were filtered with 0.5 μm glass fiber membrane (47 mm, Advantec Toyo Roshi Kaisha Ltd., Japan) to remove the particles. The pH of the 1-L filtered sample was adjusted to ~3 by spiking FA and then 0.2 g Na_2EDTA was added for the chelating of metals. Twenty nanogram of $^{13}C_3$-caffeine was spiked into the samples as surrogate. Samples were shaken and then let stand for 30 min for homogenization. Hydrophilic-lipophilic-balanced (HLB) cartridge (200 mg, 6 cc; Waters Corporation, MA, USA) was preconditioned by 5 mL of methanol, 5 mL of Milli-Q water and 5 mL of 0.02% FA (*v/v)* in Milli-Q water (pH ≈ 3). Then the 1-L sample was loaded into the preconditioned cartridge at a speed of 2 drops/s. After sample loading, the cartridge was washed with 5 mL of Milli-Q water (pH ≈ 3) and then vacuum-dried at ~5" Hg for 30 min. The target antibiotics was eluted by adding 5 mL methanol and the eluate was collected in a 15-mL PP centrifuge tube. The eluate was evaporated under a gentle stream of high-purity nitrogen to dryness at 40 °C. Forty ng of internal standard mixture was spiked into the tube and topped up to 0.5 mL with methanol/Milli-Q water (*v/v* = 1:1). This solution was finally transferred to amber PP GC vial and ready for instrumental analysis.

Instrumental analysis of the target analytes was performed using an Agilent 1290 Infinity liquid chromatograph (Palo Alto, CA, USA) coupled to a SCIEX QTRAP 5500 tandem mass spectrometer (Woodlands, Singapore), with an electrospray ionization

(ESI) interface operated in multiple reaction monitoring (MRM) mode. Agilent Zorbax Eclipse Plus C18 (2.1mm i.d. × 50mm L., 1.8 μm; Agilent, Palo Alto, CA, USA) was used as the analytical column. Mobile phase A consisted of Milli-Q water (0.02% formic acid (*v/v*)) and phase B of methanol (0.02% formic acid (*v/v*)). The gradient program and flowrate are shown in **Table A2**. All analytes were determined under positive MRM mode except chloramphenicol and cloxacillin which were determined under negative MRM mode (**Table A3**). The ESI parameters were set as follows: curtain gas 25 psi, collision gas "high", ion spray voltage 5,500 V, interface temperature 550 °C, nebulizer gas 50 psi and turbo gas 50 psi for positive mode; curtain gas 30 psi, collision gas "high", ion spray voltage -3,000 V, interface temperature 550 °C, nebulizer gas 40 psi and turbo gas 50 psi for negative mode.

Table A2. Gradient program and flowrate of the mobile phases.

Time	Flowrate (μL/min)	A	B
0	200	80	20
3	200	65	35
3.01	200	45	55
8	200	45	55
8.01	200	10	90
11	200	10	90
11.01	300	10	90
16	300	10	90
16.01	200	80	20
20	200	80	20

Table A3. MRM transitions for compound quantification and identification.

Compound	Precursor	Quantifier	Qualifier
Amoxicillin	366.066	349.1	113.9
Piperacillin	517.991	143	114.9
Cefalexin	347.934	106	139.9
Cefotaxime	455.894	167	124.9
Erythromycin-H_2O	716.267	82.9	158.1
Clarithromycin	748.387	590.3	158.3
Roxithromycin	837.572	679.6	158
Azithromycin	749.19	83.2	573.6
Tylosin	916.419	173.9	772.5
Sulfamethazine	278.977	186.1	124
Sulfamethoxazole	254.069	92	107.7
Sulfadiazine	250.935	92	65
Tetracycline	445.085	409.9	427
Chlortetracycline	479.191	462	444.1
Doxycycline	444.913	428	410
Oxytetracycline	461.014	426.1	443.2
Norfloxacin	319.98	302	276
Ofloxacin	361.853	318.1	261
Ciprofloxacin	332.006	314.1	288.1
Enrofloxacin	360.107	316	245
Metronidazole	171.986	81.8	42
Lincomycin	407.09	126	81.9
Meropenem	384.009	68	141.1
Trimethoprim	290.95	230	261
Chloramphenicol	320.913	152.1	34.9
Cloxacillin	434.066	292.8	389.9
$^{13}C_3$-Caffeine	198.108	139.8	-
Venlafaxine-d_6	284.132	58	-

Norfloxacin-d_5	325.123	307.1	-
Ofloxacin-d_8	370.479	326.1	-
Roxithromycin-d_7	844.743	157.9	-
Azithromycin-d_3	752.434	594.3	-
^{13}C-Erythromycin-d_3	738.318	580.2	-
$^{13}C_6$-Sulfamethazine	284.952	185.9	-
Doxycycline-d_3	448.203	431.1	-
$^{13}C_4$-Cloxacillin	438.097	296.8	-

Quality assurance and quality control

A 9-point standard calibration curve, with concentrations ranging from 0.005 to 1000 ng/mL (exact ranges used depend on the instrumental detection limits [IDLs] and measured levels of individual antibiotics), was used for the quantification of each target analyte. The regression coefficients (r) of the standard curves were all ≥ 0.999.

Matrix spike recovery experiments were carried out in 1-L filtered seawater, river water and wastewater effluent. IDLs were defined as an instrumental signal-to-noise ratio of ≥ 3:1. Method detection limits (MDLs) were then calculated as:

$$\mathrm{MDL} = \frac{\mathrm{IDL}}{\text{Matrix recovery} \times \text{concentration factor}} \quad (1)$$

Method quantitation limits (MQLs) were defined as 10/3 MDLs. The level in sample (x) was treated as ½MDL when x < MDL or ½MQL when MDL < x < MQL. Field and procedural blanks were performed in every sampling campaign and batch of experiments, and all target analytes in the blanks were found below MDLs. Procedural recovery experiments were also performed in every batch of experiments with bottles wrapped with aluminum foil for prevention of photodegradation. The recovery results

and detection limits are listed in **Tables A4 and A5**.

Table A4. Matrix spike recoveries (n = 3 for each matrix), IDLs, MDLs and MQLs of the target antibiotics.

Class	Compound	Seawater recovery	SD	River water recovery	SD	Wastewater recovery	SD	Average	SD	IDL (ng/L)	MDL (ng/L)	MQL (ng/L)
Penicillins	Amoxicillin	108%	7%	70%	8%	62%	4%	80%	25%	164.8	0.077	0.256
	Piperacillin	116%	6%	73%	5%	65%	1%	85%	27%	194.8	0.084	0.281
	Cloxacillin	120%	12%	84%	6%	76%	5%	93%	23%	9.7	0.004	0.013
Cephalosporins	Cefalexin	90%	5%	97%	13%	80%	0%	89%	9%	222.2	0.124	0.413
	Cefotaxime	120%	8%	62%	1%	55%	4%	79%	36%	44.4	0.019	0.062
Macrolides	Erythromycin-H_2O	90%	11%	80%	4%	96%	6%	89%	8%	5.6	0.003	0.01
	Clarithromycin	103%	3%	90%	11%	82%	16%	92%	11%	9.4	0.005	0.015
	Roxithromycin	96%	5%	86%	8%	74%	6%	85%	11%	7.7	0.004	0.013
	Azithromycin	97%	5%	87%	2%	91%	16%	92%	5%	79.4	0.041	0.137
	Tylosin	89%	18%	65%	13%	61%	2%	72%	15%	171.4	0.097	0.323
Sulfonamides	Sulfamethazine	92%	2%	92%	10%	89%	2%	91%	2%	111.1	0.06	0.2
	Sulfamethoxazole	81%	2%	78%	7%	80%	3%	80%	2%	71.4	0.044	0.147
	Sulfadiazine	114%	3%	104%	13%	90%	1%	103%	12%	33.3	0.015	0.049
Tetracyclines	Tetracycline	101%	10%	113%	14%	81%	2%	98%	16%	1388.9	0.686	2.288
	Chlortetracycline	110%	8%	86%	1%	77%	4%	91%	17%	98.4	0.045	0.149
	Doxycycline	101%	5%	81%	12%	85%	0%	89%	11%	20.3	0.01	0.033

	Oxytetracycline	95%	13%	111%	7%	94%	3%	100%	10%	86.7	0.045	0.151
Fluoroquinolones	Norfloxacin	105%	2%	116%	15%	76%	2%	99%	21%	176.5	0.084	0.28
	Ofloxacin	100%	4%	91%	5%	91%	15%	94%	5%	63.8	0.032	0.107
	Ciprofloxacin	105%	7%	149%	9%	83%	3%	112%	34%	217.4	0.104	0.345
	Enrofloxacin	100%	4%	119%	13%	85%	1%	101%	17%	60	0.03	0.1
Nitroimidazoles	Metronidazole	103%	8%	58%	10%	50%	0%	70%	29%	136.4	0.066	0.221
Lincosamides	Lincomycin	92%	6%	63%	7%	59%	2%	71%	18%	13.3	0.007	0.024
Carbapenem	Meropenem	55%	3%	48%	2%	42%	6%	48%	7%	283	0.26	0.865
Miscellaneous	Trimethoprim	113%	9%	95%	5%	95%	6%	101%	10%	49.2	0.022	0.073
Amphenicols	Chloramphenicol	107%	4%	125%	9%	112%	5%	115%	9%	26.3	0.012	0.041

Table A5. Procedural recoveries (PRs) and procedural blanks (PBs) of the target antibiotics (n = 12).

Class	Compound	PR	SD	PB
Penicillins	Amoxicillin	68%	11%	ND
	Piperacillin	80%	5%	ND
	Cloxacillin	92%	15%	ND
Cephalosporins	Cefalexin	80%	5%	ND
	Cefotaxime	72%	7%	ND
Macrolides	Erythromycin-H_2O	93%	28%	ND
	Clarithromycin	83%	11%	ND–<MDL
	Roxithromycin	72%	5%	ND
	Azithromycin	113%	8%	ND
	Tylosin	80%	13%	ND
Sulfonamides	Sulfamethazine	78%	4%	ND
	Sulfamethoxazole	75%	7%	ND
	Sulfadiazine	84%	16%	ND
Tetracyclines	Tetracycline	102%	16%	ND
	Chlortetracycline	94%	27%	ND
	Doxycycline	99%	24%	ND
	Oxytetracycline	118%	21%	ND
Fluoroquinolones	Norfloxacin	109%	12%	ND
	Ofloxacin	116%	21%	ND–<MDL
	Ciprofloxacin	106%	8%	ND
	Enrofloxacin	101%	13%	ND
Nitroimidazoles	Metronidazole	56%	11%	ND
Lincosamides	Lincomycin	67%	11%	ND
Carbapenem	Meropenem	60%	7%	ND
Miscellaneous	Trimethoprim	98%	26%	ND–<MDL
Amphenicols	Chloramphenicol	86%	9%	ND

Note: ND represents not detected; MDL represents method detection limit.

Risk assessment

Risk quotient (RQ) of individual antibiotics was calculated by dividing measured environmental concentration (MEC) by predicted no effect concentration (PNEC). Median and maximum levels of the individual antibiotics ever detected in either seawater or river water samples were adopted to evaluate the normal-case and worst-case scenario. PNEC values of most antibiotics were directly adopted from AMR Industry Alliance Antibiotic Discharge Targets (Tell et al., 2019), which followed the guidance of the European Chemicals Agency (2008), European Union Water Framework Directive of European Chemicals Agency (2008), European Union Water Framework Directive (2018) and OECD 201 guideline (OECD, 2011). PNEC values of antibiotics not available from this list were derived based on the ecotoxicological data gathered from ECOTOX (https://cfpub.epa.gov/ecotox/search.cfm, EPA, US), following the guidance of the European Chemicals Agency (2008) (**Table A6**). Notably, PNEC values from AMR Industry Alliance Antibiotic Discharge Targets were acquired in two target groups, namely PNEC-ENV for the aquatic environment and PNEC-AMR for AMR (**Table A7**). Thus, risk assessment for individual antibiotics was performed for these two targets, separately. Antibiotics were identified as "potential risk" when RQ values ≥ 1 while "no risk" when RQ values < 1.

For those identified as "potential risk" in the worse-scenario case, probabilistic risk assessment was further conducted to reveal the possibilities that the potentially risky antibiotics were likely to cause adverse effects. Linear regression between cumulative probabilities (p) and MEC on log scale was plotted. The probabilities (100-p)% of the

samples posing risks were then determined by substituting the log values of PNEC in the linear equations derived (Tsui et al., 2014).

Table A6. Derivation of PNEC-ENV of antibiotics that were not available from AMR Industry Alliance Antibiotic Discharge Targets (Tell et al., 2019).

Compound	AF	PNEC-ENV (µg/L)	Taxonomic group	Concentration (mg/L)	Effect	Endpoint	Reference
Amoxicillin	50	530	Fish	128	Mortality	NOEC	(Oliveira et al., 2013)
			Algae	250	Population	NOEC	(De Orte et al., 2013)
			Invertebrates	26.5	Development	EC_{05}	(Carballeira et al., 2012)
Cloxacillin	1000	61	Algae	61	Population	EC_{10}	(Kusk et al., 2018)
Sulfamethazine	1000	1	Algae	1	Population	NOEC	(Yang et al., 2008)
			Crustacean	31.4	Intoxication	EC_{50}	(Jung et al., 2008)
Chlortetracycline	50	0.24	Algae	0.5	Population	NOEC	(Yang et al., 2008)
			Crustacean	111.2	Intoxication	EC_{50}	(Kim et al., 2010)
			Fish	0.012	Growth	NOEL	(Koeypudsa et al., 2005)
Metronidazole	100	125	Algal	12.5	Population	EC_{50}	(Lanzky and Halling-Sorensen, 1997)
			Crustacean	100	Mortality	NOEC	(Lanzky and Halling-Sorensen, 1997)
Chloramphenicol	100	1	Algal	0.1	Population	IC_{50}	(Sanchez-Fortun et al., 2009)
			Crustacean	2	Intoxication	NOEC	(Williams et al., 1992)
			Mollusks	74.29	Development	EC_{50}	(Davis and Hidu, 1969)

Note: Ecotoxicology data were generated from ECOTOX (https://cfpub.epa.gov/ecotox/search.cfm, EPA, US) with priority given to chronic effects of different trophic levels. AF values were determined following ECHA technical guide.

Table A7. Values of PNEC-ENV (µg/L) and PNEC-AMR (µg/L) for the target antibiotics adopted from AMR Industry Alliance Antibiotic Discharge Targets (Tell et al., 2019) and Table A6. NA represents not available.

Compound	PNEC-ENV	PNEC-AMR
Amoxicillin	530	0.25
Ampicillin	0.87	0.25
Piperacillin	NA	0.5
Cloxacillin	61	0.13
Cefalexin	0.08	4
Ceftazidime	1.3	0.5
Cefotaxime	0.1	0.13
Erythromycin-H_2O	0.5	1
Clarithromycin	0.08	0.25
Roxithromycin	6.8	1
Azithromycin	0.02	0.25
Sulfamethazine	1	NA
Sulfamethoxazole	0.6	16
Sulfadiazine	13	NA
Tetracycline	3.2	1
Chlortetracycline	0.25	NA
Doxycycline	NA	2
Oxytetracycline	18	0.5
Norfloxacin	120	0.5
Ofloxacin	10	0.5
Ciprofloxacin	0.57	0.06
Enrofloxacin	1.9	0.06
Metronidazole	125	0.13
Lincomycin	0.81	2
Meropenem	1.5	0.06
Trimethoprim	100	0.5
Chloramphenicol	1	8

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