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Select antibiotics in leachate from closed and active landfills exceed thresholds for antibiotic resistance development

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Abstract

Though antibiotic resistance (ABR) represents a major global health threat, contributions of landfill leachate to the life cycle of antibiotics and ABR development are poorly understood in rapidly urbanizing regions of developing countries. We selected one of largest active landfills in Asia and two landfills that have been closed for 20 years to examine antibiotic occurrences in leachates and associated hazards during wet and dry season sampling events. We focused on some of the most commonly used human antibiotics in Hong Kong, one of the most populous Asian cities and the fourth most densely populated cities in the world. Seven antibiotics (cephalexin [CLX][1], chloramphenicol [CAP], ciprofloxacin [CIP], erythromycin [ERY], roxithromycin [ROX], trimethoprim [TMP], sulfamethoxazole [SMX]) were quantitated using HPLC-MSMS generally following previously reported methods. Whereas CLX, CAP, ROX and SMX in leachates did not exceed ABR predicted no effect concentrations (PNECs), exceedances were observed for CIP, ERY and TMP in some study locations and on some dates. In fact, an ABR PNEC for CIP was exceeded in leachates during both sampling periods from all study locations, including leachates that are directly discharged to coastal systems. These findings highlight the importance of developing an advanced understanding of pharmaceutical access, usage and disposal practices, effectiveness of intervention strategies (e.g., leachate treatment technologies, drug take-back schemes), and contributions of landfill leachates to the life cycle of antibiotics and ABR development, particularly in rapidly urbanizing coastal regions with less advanced waste management systems than Hong Kong.

Key words: urbanization; antibiotic resistance; predicted no effect concentration; landfill leachate; Hong Kong

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[1] cephalexin (CLX), chloramphenicol (CAP), ciprofloxacin (CIP), erythromycin (ERY), roxithromycin (ROX), trimethoprim (TMP), sulfamethoxazole (SMX), predicted no effect concentrations (PNECs), antibiotic resistance (ABR), Environmental Protection Department (EPD), Shuen Wan (SW), Pillar Point Valley (PPV), West New Territories Landfill (WENT), solid phase extraction (SPE).

1. Introduction

By 2050 the global population is predicted to reach 9.8 billion people, many of which will be concentrated in Asia. Whereas Asia contributed 55% to the global population in 2014, 22 megacities are expected in Asia by 2030. Higher population densities are also observed in this region; for example, Hong Kong is presently the fourth most densely populated city in the world. Such population densities correspondingly result in a concentration of urban consumption of natural resources, including food, energy and water, and consumer products. In fact, in many urban areas of Asia and other regions, access to and consumption of consumer chemicals, including medicines, are occurring more rapidly than implementation of public health and environmental interventions, including advanced wastewater and solid waste management (Brooks 2018). This couples with dramatically variable standards of waste management practices and treatment technologies among developed and developing regions, differential implementation and protection of environmental quality from risks posed by contaminants of historical and emerging concern is inevitable.

In recent years, pharmaceuticals have received attention as contaminants of emerging concern because they represent indicators of an urbanizing environment and water cycle (Brooks 2014). Antibiotics, in particular, have been examined as environmental contaminants due to associated risks presented to aquatic and terrestrial ecosystems (Brooks et al. 2008). For example, Brain et al. (2008) examined adverse effects of antibiotics to aquatic plants and algae, and identified likely sensitive species resulting from increased evolutionary conservation of therapeutic targets for a number of antibiotic classes. Antibiotic occurrence in the environment has also received heightened attention due to influences on the development of antibiotic resistance (ABR), which is now spreading (Qiao et al., 2018) and represents a leading threat to global public health (World Health Organization, 2015). For example, influences of antibiotics in the environment on the development of ABR microorganisms was recently identified as a priority global research need (Boxall et al. 2012, Rudd et al. 2014).

Numerous studies have reported the environmental introduction of antibiotics and other pharmaceuticals from manufacturing processes (Larsson, 2014), and following human use through discharge of municipal wastewater and land application of biosolids, and veterinary use in terrestrial agriculture and aquaculture operations (Qiao, et al. 2018). Antibiotics and other medicines may also enter the environment from solid waste management processes, which has been the subject of several studies. For example, Holm et al. (1995) observed five sulfonamide compounds from 40 – 1600 μg/L in ground water influenced by an unlined landfill in Denmark. Masoner, et al. (2016a, b) found that contaminants of emerging concern, including antibiotics, were commonly found in raw and final leachate in US landfills. Wu et al. (2015) examined levels of 20 antibiotics in the leachate from two refuse transfer stations and one landfill site in Shanghai. Despite relatively elevated concentrations for all reported samples, these levels were generally higher in leachate from transfer stations than the landfill. Regarding the relationship between the age of the landfill and concentration of antibiotics in associated leachate plumbs, Wu et al. (2017) found that with the exception of sulfamethazine, lower levels were observed from older landfills. Whereas such findings appeared to conflict with a conclusion by Yu et al. (2016) that the levels of sulfapyridine and sulfadiazine increased with the age of the landfill, it was in fact not. This was because the oldest landfill studied by Yu et al. (2016) was just 6 years in age, which would be considered a middle-aged landfill by Wu et al. (2017). Beyond these recent studies, the environmental occurrence and risks of pharmaceuticals in general (Kristofco and Brooks 2017, Saari et al. 2017) and antibiotics in particular are not well studied in many parts of Asia (see Table 1). Further, how landfill leachate, an important type of wastewater effluent, but highly variable in its compositions, contributes to the life cycle of antibiotics is still largely not understood.

In the present study, we selected Hong Kong, one of the most populous cities in Asia, for an initial study to determine whether commonly used human antibiotics (cephalexin [CLX], chloramphenicol [CAP], ciprofloxacin [CIP], erythromycin [ERY], roxithromycin [ROX], trimethoprim [TMP], and sulfamethoxazole [SMX]) were present in and/or differed among leachates of closed and active landfills during wet and dry seasons. Specifically, the Shuen Wan (SW) and Pillar Point Valley (PPV) landfills were selected because these restored facilities were closed in 1995 and 1996, respectively, whereas we studied the West New Territories Landfill (WENT) because it represented one of the largest active landfills in Asia, and then compared our findings to previous research (Table 1) that also examined these target analytes. We further identified whether presence of antibiotics exceeded proposed no effect concentrations for the development of ABR microorganisms.

2. Methods

2.1 Study locations

One of the largest active landfills in Asia, WENT opened in 1993, has a designed capacity of 61 million m^3, and is currently receiving domestic, industrial, commercial and construction waste (Environmental Protection Department (EPD), 2015b). Leachate from WENT is temporarily stored and aerated in leachate lagoons on site and then is piped for stripping of ammonia at 1000ºC and then treated in a biological sequencing batch reactor. Treated leachate is connected to the municipal sewer and discharged to North Western Water Control Zone in Hong Kong via a submarine outfall (EPD, personal communication, 10 August 2016).

PPV started operation in 1983 and was closed on 1996. A total of 13 million tons of domestic, construction, commercial and industrial wastes were landfilled there. Restoration started in 2004 when capping, treatment facilities, membrane, gas collection, surface drainage were added (EPD, 2015a). PPV contains an on-site ammonia stripping facility very similar to

WENT. Treated leachate is transferred to the Pillar Point Sewage Treatment Works for chemical enhanced primary treatment before discharging to the North Western Water Control Zone via a submarine sewage outfall (EPD, personal communication, 10 August 2016). While requests for raw leachate samples from PPV were made, EPD explained that only treated leachate could be provided for study.

SW landfill started operation ten years earlier than PPV (in 1973), was closed in 1995, and then restoration started in 1996. It received a total of 15 million tons of domestic waste, commercial, industrial and construction waste during its operation (EPD, 2015a). Collected raw leachate is pumped to the Tai Po sewage treatment work for secondary treatment and the treated wastewater will be discharged to Victoria Harbor (Phase Two) Water Control Zone under the Tolo Harbor Effluent Export Scheme (EPD, personal communication, 10 August 2016). No treated leachate sample was available because there is no leachate treatment facility on-site. This restored landfill is now used as a golf driving range. It is important to note that prior to commissioning of a sewage sludge incinerator during 2015 (EPD, 2016), all sewage sludge was landfilled in Hong Kong. As a result, both PPV and SW received sewage sludge throughout their life spans and WENT continued to receive sewage sludge until April 2015. Figure 1 shows locations of each of these landfills.

Figure 1. Study locations included an active and two closed landfills in Hong Kong.

2.2 Sample collection

Because infiltration from precipitation influences production of landfill leachate, we selected June and January for sampling in Hong Kong, which typically corresponds to the highest annual rainfall in June and the lowest annual rainfall in January. Duplicate leachate samples of each location were collected from PPV, SW, and WENT on 26 June 2015 and 12 Jan 2016. In all cases, pre-cleaned 4L amber colored glass bottles conforming to Environmental Protection Agency (EPA) contaminant free standard were used to hold samples. In the field, glass bottles were sample-rinsed three times immediately before sample collection. For the field blank, one pre-cleaned 4L amber glass bottle was exposed at the raw leachate collection site (in the June 2015 campaign) and another at the treated leachate sampling site (in the January 2016 campaign).

2.3 Sample extraction

A one liter aliquot of each sample were sequentially filtered by 0.45 µm glass fiber filter and 0.22 µm nylon membrane using a standard Buchner funnel and flask filtration set up. Before extraction, for each 500 ml of filtered sample, 5 ml of 5% (w/v) Na_2EDTA was added as a chelating agent. 5M formic acid was added to adjust the solution to pH 3.0-3.5. The filtered samples were then extracted with Oasis Hydrophilic–Lipophilic Balanced (HLB) solid phase extraction (SPE) cartridges and SPE manifold (Sigma-Aldrich Co. USA). Target analytes in each cartridge were eluted with methanol for determination by a high-performance liquid chromatograph–tandem mass spectrometer (HPLC–MS/MS).

2.4 Chemicals and standards

All antibiotics standards (TMP, CLX, CIP, SMX, CAP, ERY and ROX) were procured from Sigma-Aldrich with purities of ≥99.5% for CLX, SMX and ERY, ≥98% for TMP, CIP and CAP, and ≥90%for ROX. Individual stock solutions of antibiotics were prepared at concentrations of 1000 µg/l by dissolving appropriate amounts of antibiotics in methanol and stored in the dark at -20°C. Nine concentrations of mixed working solutions, namely, 500, 250, 125, 62.5, 31.25, 15.625, 7.813, 3.906 and 0.00 ng/ml, were freshly prepared in Milli-Q water. Caffeine -$^{13}C_{12}$, purchased from Aldrich Co., was used as an internal standard. These methods generally followed those previously reported (Minh et al. 2009).

2.5 Instrumental analysis

After SPE, samples were evaporated by a gentle nitrogen stream to nearly dry, then 2 ml of Milli-Q water plus the internal standard, $^{13}C_{12}$ caffeine was added. Samples were then vigorously shaken and centrifuged (4000 rpm) for 10 minutes. Only the clear solution on the top was taken and transferred to an amber auto-sampler vial and injected into XBridgeTM

C18 column (Waters Corp., 2.1 mm i.d. x 50 mm length, 5 um) with a guard column for HPLC–MS/MS with electrospray ionization determination at Hong Kong Baptist University. Determination was carried out by operating in positive mode for all antibiotics except for CAP, which was determined by the negative mode. An aliquot of 5 µl of extract was automatically taken for injection in the mentioned column.

Milli-Q water acidified with 10 mM formic acid and methanol acidified with 10 mM formic acid were used as mobile phases at a flow rate of 300 µl/min. The gradient program started at 10% methanol and was increased to 90% methanol in 10 minutes, before reverting to the original conditions at the thirteenth minute for the next sample. Antibiotics were identified by comparing retention times of their product ions in each sample (see Table 2) with the retention times of standards allowing discrepancy of no more than 0.3 min. Concentrations of target analytes in samples were auto-calculated (based on the ratio of peak area of target compounds and the internal standard) by the built-in standard calculation curve program of the computer.

2.6 Quality control

All glass bottles used in the sampling conformed to USEPA's contaminant free standard. They were pre-cleaned with laboratory grade detergent, tap water, methanol and then Milli-Q water before using them to hold field samples. Collected samples were stored in ice-cube filled coolers and taken back to the laboratory immediately after sampling. Calibration curves at 9 calibration points (500, 250, 125, 62.5, 31.3, 15.6, 7.8, 3.9 and 0.00µg/l) were constructed and linearity was confirmed with $R^2 > 0.99$. Procedural recovery rates of the target analytes were obtained by analyzing leachate samples spiked with 100ng of the 7 antibiotics and 120 ng of internal standards (Table 2). Our recoveries are very similar to those previously reported in sewage effluent by Minh et al. (2009) and comparable to those reported in Gulkowska et al. (2007).

Table 2. Instrumental parameters and percent recovery of seven antibiotics in landfill leachates from Hong Kong.

Antibiotic	Precursor ion	Product ion	Retention time (min.)	Recovery
CLX	348.1	158.3; 174.2	6.2	98%
CAP	321	152.0; 257.1	7.6	99%
CIP	331	314	5.3	90%
ERY	716.6	158.2; 558.7	10.3	102%
ROX	837.8	158.5; 679.8	10.9	99%
SMX	254.1	156.0; 108.0	6.4	92%
TMP	291.2	123.0; 261.2	5.5	104%

Notes: CLX: cephalexin; CAP: chloramphenicol; CIP: ciprofloxacin; ERY: erythromycin; ROX: roxithromycin; SMX: sulfamethoxazole; TMP: trimethoprim.

3. Results and Discussion

All 7 target antibiotics were detected in all samples and generally in the following order of highest to lowest concentrations: CIP>ERY>CLX>TMP>SMX>ROX>CAP (Table 3). As noted above, we initially selected January and July sampling dates because these periods typically represent months with lowest and highest historical rainfall, respectively, in Hong Kong. Such trends prevail during the present study with 84.3 mm and 158.6 mm total rainfall observed in the seven days preceding our sampling events in January and June, respectively. However, precipitation on the day immediately preceding January and June sampling events were almost identical at 30.7 mm and 28.5 mm, respectively (Hong Kong Observatory, 2016). The Related-Samples Wilcoxon test results ($p=0.04$) showed that concentrations of antibiotics across sites were significantly elevated during January, compared to June sampling events (Table 3). Whether such observations resulted from decreased dilution of leachate following lower precipitation in January compared to June is not known but highlights the need to better understand seasonal precipitation influences on antibiotic levels in leachate discharges to the environment.

Table 3. Mean (duplicate) concentrations of antibiotics in leachate plumes of two restored and one active landfill in Hong Kong.

	SW Raw		PPV Treated		WENT Raw		WENT Treated	
Analytes (ng/L)	January	June	January	June	January	June	January	June
CLX	481	131	505	90	1511	600	502	344
CAP	150	163	83	81	92	176	105	93
CIP	793	1061	898	351	3410	1756	1259	751
ERY	1104	2048	906	177	2133	3001	903	304
ROX	521	177	109	9	435	241	140	77
SMX	762	31	126	33	385	52	135	78
TMP	376	747	24	48	231	939	73	151

Notes: CLX: cephalexin; CAP: chloramphenicol; CIP: ciprofloxacin; ERY: erythromycin; ROX: roxithromycin; SMX: sulfamethoxazole; TMP: trimethoprim. SW: Shuen Wan restored landfill; PPV: Pillar Point Valley restored landfill: WENT: West New Territories active landfill.

We also observed generally elevated levels of these 7 antibiotics in raw leachate from the active WENT landfill and the restored SW landfill than treated leachate from WENT and the restored PPV landfill (Table 3; Figures 2, 3). Among the sampling locations, highest total concentrations of all seven antibiotics were generally observed in following order: WENT raw > SW raw > WENT treated > PPV treated (Table 3; Figures 2, 3) with antibiotic concentrations in raw leachate were significantly greater than that in treated leachate for both seasons (Mann Whitney U test: p=.000). But antibiotics in leachate from active landfills were significantly greater than in closed landfills in the wet seasons only (Mann Whitney U test: p=.007) and not in the dry seasons. The inability to reject the null hypothesis in the last case is likely due to the elevated levels of antibiotics in the raw leachate from SW relative to those in the treated leachate from WENT in the dry seasons. Thus, it appeared that leachate treatment represented a more important factor in determining antibiotics concentrations in leachate than whether the landfill was active or not.

We were not able to collect treated leachate samples from SW or raw leachate from PPV; however, treated leachate from PPV contained some of the lowest levels of the target analytes

(Table 3). Yet, from the above comparisons, it is possible that such observations may have resulted from the availability of ammonia stripping (at 1000ºC) at PPV for treating leachate. However, the low concentration of target analytes in leachate of PPV might also have resulted from its shorter duration of operation. Further studies are required to understand the effectiveness of various treatment technologies for antibiotics.

We consistently observed lower levels among these targeted antibiotics in treated leachate samples from WENT compared to raw untreated leachate on both sampling dates (Table 3). Such observations suggest some reduction of antibiotic introductions to coastal waters by the existing leachate treatment system at WENT. However, it is also important to note that we also observed higher concentrations of four antibiotics (CAP, ROX, SMX, TMP) in SW raw leachate than WENT raw leachate during the January sampling event (Table 3). These observations are interesting because, as noted above, the SW landfill stopped receiving waste in 1995 and subsequently went through a restoration process, whereas the WENT landfill remains one of the largest active landfills in Asia. These results indicate that future studies are needed to better understand contributions from both active and closed landfills to the life cycle of antibiotics in the environment and the potential development of antibiotic resistance.

Though the presence of pharmaceuticals in the environment has received attention in Asia for some time (Richardson et al. 2005, Chen et al. 2012, Bu et al. 2013, Lui and Wong 2013, Wang et al. 2015), there remains limited information on the occurrence of antibiotics in leachates from closed and active landfills, particularly in Asia and other rapidly urbanizing regions of less developed countries. However, Lu et al. (2016) studied 26 pharmaceuticals in leachate plumes of four landfills in Taichung, Taiwan, a city with ~2.8 million inhabitants.

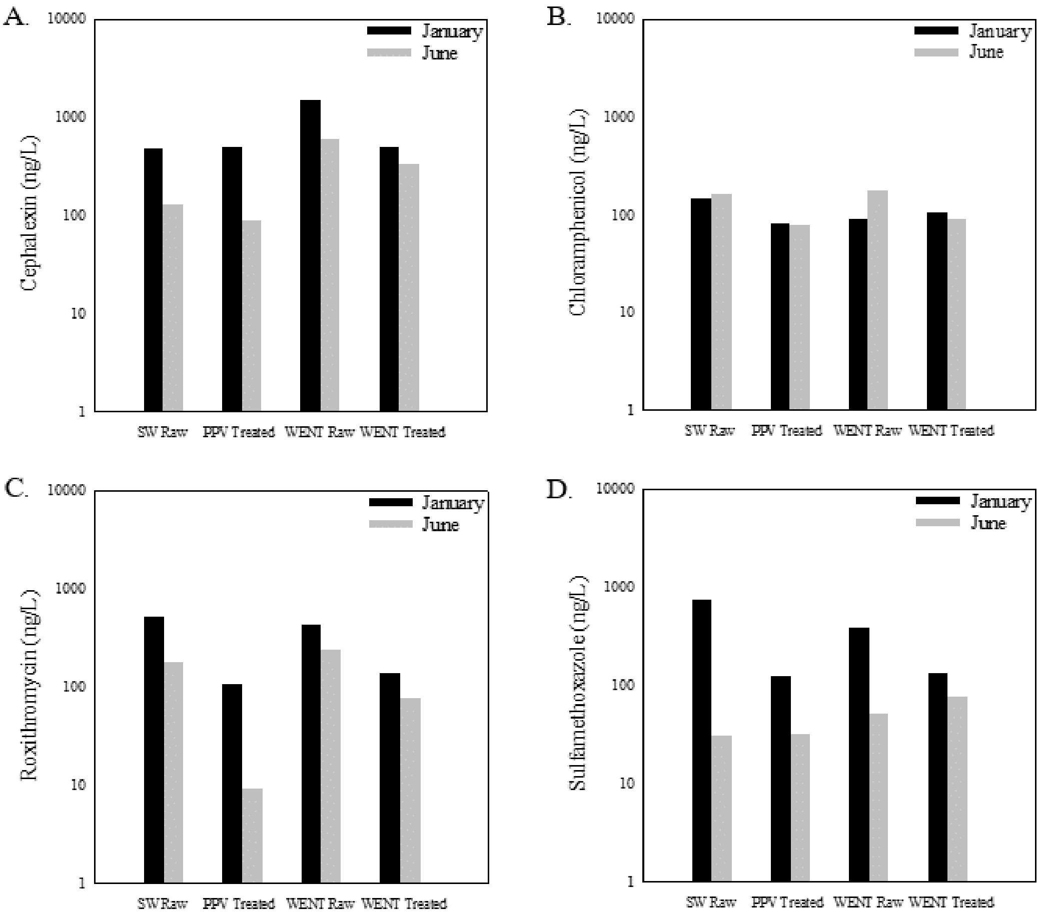

Figure 2. Mean (duplicate) concentrations of four antibiotics (cephalexin, chloramphenicol, roxithromycin, sulfamethoxazole) not exceeding predicted no effect concentrations for antibiotic resistance development in raw and treated leachate plumes of two restored and one active landfill in Hong Kong. SW: Shuen Wan restored landfill; PPV: Pillar Point Valley restored landfill: WENT: West New Territories active landfill.

Of the 26 pharmaceuticals studied by Lu et al. (2016), two antibiotics, the macrolide ERY and the sulfonamide SMX, were also examined in the current study. We observed markedly higher (by an order of magnitude) levels of these antibiotics compared to findings in Taichung and other reviewed studies but not as high as the highest measured in the leachate plume from a 6-year-old landfill in Shanghai (Yu et al., 2016). If landfills in Taichung and Hong Kong are comparable (and thus the rates of degradation of the two antibiotics through time are similar), then elevated levels of these two antibiotics in the leachate plumes from Hong Kong may suggest greater consumption and disposal of unused medicines as solid waste in a more highly populated city (e.g., Hong Kong population is ~7.3 million).

Unfortunately, we could not locate local production and consumption data for antibiotics in Hong Kong and Taichung. Thus, such conjectures highlight the importance of developing an advanced understanding of human pharmaceutical consumption and disposal patterns in Asian cities.

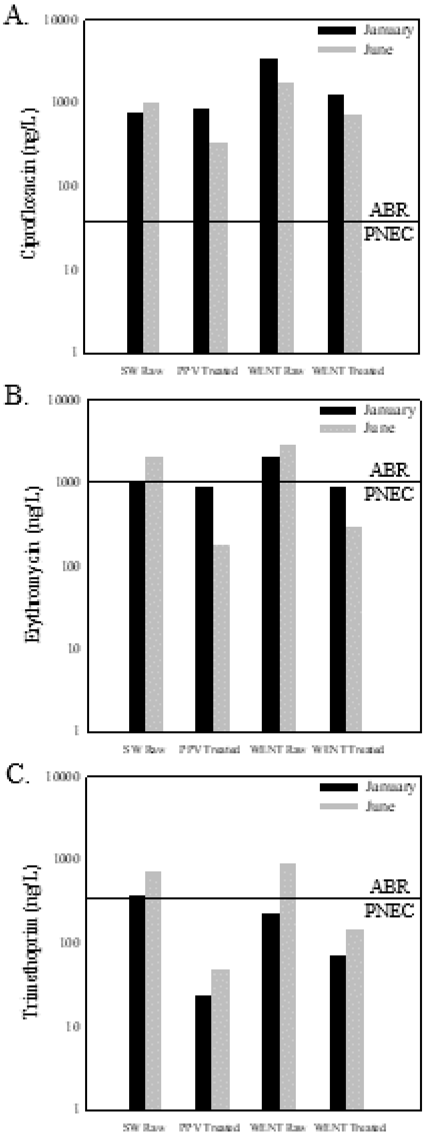

Figure 3. Mean (duplicate) concentrations of three antibiotics (ciprofloxacin, erythromycin, trimethoprim) exceeding predicted no effect concentrations (PNEC) for antibiotic resistance (ABR) development in raw and treated leachate plumes of two restored and one active landfill in Hong Kong. Solid horizontal lines indicated ABR PNEC values (Bengtsson-Palme and Larsson 2016). SW: Shuen Wan restored landfill; PPV: Pillar Point Valley restored landfill: WENT: West New Territories active landfill.

In Europe, the Landfill Directive 1999/EC stipulates that leachate composition be sampled at quarterly and half-yearly intervals for active and closed landfills, respectively, and that leachate has to be properly treated before being discharged to surface waters (European Council, 1999). However, no specific discharge limits for antibiotics are stated in the Directive. Similarly, in the US, there is also no Federal specific discharge standard imposed for leachate. Rather, the focus falls on concentrations of pollutants at the point of exposure (e.g., receiving system). However, the EPA will not approve a landfill design unless it can be ensured that the quality of the water at the upper most aquifer within 150 meters from the boundary of the landfill comply with the 24 maximum contaminant levels (EPA, 1993). Here again, no pharmaceutical compounds are included in this list. In Hong Kong, the managing authority of landfills (i.e., EPD) monitors landfill leachate for 26 parameters, which include BOD, COD, total and individual metals (e.g., Cd, Hg), total nitrogen, total phosphorous and phenols (EPD, personal communication on 10th August, 2016). Yet again, pharmaceutical compounds are not currently included in leachate quality monitoring programs for Hong Kong. Whether antibiotics should be included in leachate quality monitoring should be informed by environmental occurrence and associated risks to public health and the environment.

Translating environmental observations of pharmaceuticals to hazards and risks has followed traditional environmental risk assessment regulatory frameworks and has more recently received advanced study (Brooks 2014, 2018; Brooks and Steele 2018). For example, Kristofco and Brooks (2017) and Saari et al. (2017) employed probabilistic environmental hazard assessment for antihistamines and calcium channel blockers, respectively. In addition to considering traditional ecotoxicology datasets, these studies specifically employed a therapeutic hazard value approach with environmental observations because aquatic hazards associated with therapeutic (and side effect) mechanisms of action often occur below concentrations eliciting ecotoxicological responses (e.g., survival, growth) in standardized testing methods (Brooks 2014). Bengtsson-Palme and Larsson (2016) recently derived minimal selective concentrations and predicted no effect concentrations (PNECs) for antibiotics in an attempt to identify thresholds associated with development of ABR. This approach effectively complements previous ecotoxicology efforts with antibiotics (Brain et al. 2008, Brooks et al. 2008, Brausch et al. 2012) because, much like therapeutic hazards, development of ABR can be elicited by antibiotics at lower levels than thresholds detected by standardized ecotoxicology assays (Bengtsson-Palme and Larsson 2016).

In the current study, we employed previously derived ABR PNECs by Bengtsson-Palme and Larsson (2016) to aid interpretation of our observations of 7 antibiotics in landfill leachates from Hong Kong. Though ABR PNECs for CLX, CAP, ROX and SMX were not exceeded in any study location during the January and June sampling events (Figure 2), exceedances of ABR PNECs for CIP, ERY and TMP were identified. Specifically, TMP exceeded an ABR PNEC of 500 ng/L in raw leachate from the restored SW and the active WENT landfills, but only during the January sampling event (Figure 3; Table 3). Similarly, an ABR PNEC (1000 ng/L) for ERY was exceeded in raw leachate from SW and WENT during both sampling events and were approached in treated leachate from PPV and WENT in January (Figure 3; Table 3). Such exceedances are noteworthy because Minh et al. (2009) previously reported ambient levels (e.g., 1900 ng/L) of ERY in Victoria Harbor of Hong Kong exceeding the ABR PNEC of 1000 ng/L. However, ABR PNEC exceedances for CIP were more profound in the current study. All CIP measurements from each study location on both sampling dates markedly exceeded an ABR PNEC for CIP of 64 ng/L (Figure 3; Table 3). This included treated landfill leachate discharged directly to coastal waters of Hong Kong. This is noteworthy because despite quoting the highest reported levels in Table 1 for comparison, there were only two incidences of ABR PNEC exceedance among the 8 studies reviewed. Herein, it is important to note that other regions of Asia, where 22 megacities will appear by 2030, have not yet implemented environmental monitoring systems and solid and liquid waste treatment technologies as advanced as Hong Kong.

Among the three antibiotics exceeding ABR PNECs, CIP has the shortest history of use as it was approved by US Food and Drug Administration only in 1987 (eMedExpert, 2016). Because there is usually a lag time of several years for new drugs to penetrate the market, the amount of waste CIP disposed in the three landfills studied could be expected in this order: WENT > SW > PPV. Interestingly, this order generally corresponded with the order of leachate concentrations and magnitude of ABR PNEC exceedances for CIP among landfills we examined. It is also important to note that the use of CIP appears to be rapidly gaining popularity. Though we could not access CIP consumption data for Hong Kong, according to data from the Drug Office of Hong Kong Government, in 2015 there were just 3 newly registered products containing CIP in Hong Kong. However, by 1 December 2017, there were already 99 registered products containing CIP (Drug Office, 2017). In fact, these observations underscore the importance of initiating future studies to understand urban metabolism, pharmaceutical access and use patterns, and ABR development in rapidly urbanizing coastal regions, which have historically received less study than freshwater systems (Gaw et al. 2014), particularly in developing countries (Kookana et al. 2014).

4. Limitations and Conclusions

Here we examined a much neglected but critically important issue in environmental management. We identified seven antibiotics in leachates of one of the largest active landfills in Asia, and in two landfills that have been closed for 20 years. Our results are also consistent with other similar studies in that older landfills while may have lower antibiotic concentrations, the release is still significant in total quantities. Such observations suggest that some antibiotics are recalcitrant under methanogenic conditions of active and closed landfills, which represent important sources to the life cycle of antibiotics in the environment. We further observed ABR PNEC exceedances of CIP, ERY and TMP in select leachates; in fact, CIP exceedances were observed in all samples examined in this study and in one recent case in Shanghai. It is noteworthy that CIP in leachate from Hong Kong is discharged directly to coastal surface waters. Such observations suggest that antibiotics should be included in leachate quality monitoring of landfills, and implementation of intervention approaches (e.g., advanced treatment technologies, drug take-back programs for unused household medicines) should be considered in Hong Kong.

It is important to note that we only examined a limited number of antibiotics over a relatively short period of time. More (longitudinal) research is needed to understand the extent to which such exceedances of ABR PNECs to the coastal areas of Hong Kong, prior to implementing potential interventions. Research should also be conducted to understand antibiotic levels in leachates from other waste facilities, such as refuse transfer stations where most of the municipal solid waste is temporarily stored before being landfilled in Hong Kong. Further, additional research is needed to understand antibiotic consumption, antibiotic contributions from landfills to the environment and ABR development, particularly in rapidly urbanizing coastal regions of developing countries, which are increasingly accessing medicines and concentrating chemical use with less developed environmental monitoring systems and solid waste management and treatment technologies than currently implemented in Hong Kong.

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Table 1. Select studies on antibiotics in landfill leachates and associated exceedances of thresholds for development of antibiotic resistance.

Year, location	No. of landfills	Nature of landfills	Engineering systems for leachate	Leachate will be treated before discharging into the environment?	Analytes	Antibiotics tested, levels[1] & PNEC[2] (ng/L)	Sources
2011, 16 States in US	19 (12 municipal + 7 private)	Active, MSW, non-hazardous commercial and industrial waste	Leachate collection & recovery systems in place	Yes, in wastewater treatment plants.	202 contaminants of emerging concern (CECs) in untreated fresh leachate	sulfadimethoxine (650; n.a.), sulfamethizole (1040; n.a.), sulfamethoxazole (260; 16000), trimethoprim (190; 500)	Masoner, et al. 2016a
2012, 12 States in US	22 (16 municipal + 6 private)	16 active, 6 closed in the 1980s and 1990s.	Leachate collection & recovery systems in place for the active landfills only	Leachate from 12 landfills will be treated with on-site facilities; leachate from 4 be treated in wastewater treatment plants	190 CECs in final leachate (after storage or on-site treatment)	sulfadimethoxine (1310; n.a.), sulfamethizole (2080; n.a.)	Masoner, et al. 2016b
2000-2002, Indiana State in US	One	Closed, MSW, industrial & medical waste	Unlined	Not specified	6 organic compounds, 16 waste indicator compounds, 2 PAHs, 3 flame retardants, 2 plasticizers, 3 hormones, 6 pharmaceuticals & metabolites.	n.a.	Buszka et al., 2009.
2014, Shanghai, China	One	Active, MSW.	Not specified	Not specified	20 antibiotics & 6 antibiotic resistant genes (ARGs)	sulfadiazine (83.6; n.a.), sulfapyridine (2210.6; n.a.), sulfamethoxazole (72.3; 16000), sulfathiazole (2186.0; n.a.), sulfamerazine (659.2; n.a.), sulfamethazine (659.2; n.a.), sulfaquinoxaline (29.3; n.a.), norfloxacin (25.9; 500), **ciprofloxacin (77.3; 64),** enrofloxacin (29.9; 64), ofloxacin (211.0; 500), tetracycline (0.2; 1000),	Wu et al. 2015

						oxytetracycline (0.7; 500), doxycyclinehyclate (n.d; n.a.), chlorotetracycline (n.d.; n.a.), **erythromycin (4963.2; 1000)**, roxithromycin (101.2; 1000), chloramphenicol (33.1; 8000), thiamphenicol (0.6; 1000), florfenicol (0.8; 2000).	
2015-6, Shanghai, China	One	Closed and active phases, MSW.	Not specified	Not specified	11 antibiotics, 11 metals and 18 ARGs	**oxytetracycline (1070; 500),** tetracycline (90.4; 1000), deoxytetracycline (472; n.a.), sulfamethazine (38.1; n.a.), sulfadiazine (450; n.a.), sulfamethoxazole (222; 16000), trimethoprim (182; 500), roxithromycin (215; 1000), erythromycin-H_2O (1620; n.a.) cephalexin (28.2; 4000), amoxicillin (151; 250).	Wu et al., 2017
2015, Shanghai, China	One	Closed and active phases, MSW.	Not specified	Not specified	7 antibiotics, 3 ARGs and 4 mobile genetic elements (MGEs)	sulfadiazine (15566; n.a.), sulfapyridine (1263; n.a.), sulfathiazole (632; n.a.), sulfamethoxazole (962; 16000), sulfamerazine (1192; n.a.), sulfamethazine (1099; n.a.), sulfaquinoxaline (n.d.; n.a.).	Yu et al., 2016
1990s, Denmark	One	Closed, household waste	No leachate collection system	Not specified, but likely untreated	5 antibiotic compounds & 8 other compounds	sulfanilamide (170000; n.a.) sulfaguanidine (1600000; n.a.) sulfadiazine (480000; n.a.) sulfadimidine (900000; n.a.) sulfamethizole (310000; n.a.)	Holm et al. 1995
2010s, Taichung, Taiwan	Four	3 closed, 1 active; MSW and incinerator ash	Leachate collection in place	All leachate will be treated	2 antibiotics and 24 other pharmaceuticals	sulfamethoxazole (n.d.; 16000) erythromycin (242.4; 1000)	Lu et al., 2016

Notes: font bolded to indicate exceedance of PNEC,

[1] Reporting limits or highest reporting limits

[2] Bengtsson-Palme & Larsson (2016)