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Hazard/Risk Assessment

Occurrence of Pharmaceutically Active Compounds and Potential Ecological Risks in Wastewater from Hospitals and Receiving Waters in Sri Lanka

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Abstract: The presence of pharmaceutically active compounds (PACs) in the environment and their associated hazards is a major global health concern; however, data on these compounds are scarce in developing nations. In the present study, the existence of 39 non-antimicrobial PACs and six of their metabolites in wastewater from hospitals and adjacent surface waters in Sri Lanka was investigated from 2016 to 2018. The highest amounts of the measured chemicals, including the highest concentrations of atorvastatin (14,620 ng/L) and two metabolites, mefenamic acid (12,120 ng/L) and *o*-desmethyl tramadol (8700 ng/L), were detected in wastewater from the largest facility. Mefenamic acid, gemfibrozil, losartan, cetirizine, carbamazepine, and phenytoin were detected in all the samples. The removal rates in wastewater treatment were 100% for zolpidem, norsertaline, quetiapine, chlorpromazine, and alprazolam. There was substantial variation in removal rates of PACs among facilities, and the overall data suggest that treatment processes in facilities were ineffective and that some PAC concentrations in the effluents were increased. The estimated risk quotients revealed that 14 PACs detected in water samples could pose low to high ecological risk to various aquatic organisms. Compounds such as ibuprofen, tramadol, and chlorpromazine detected in untreated and treated wastewater at these facilities pose a high risk to several aquatic organisms. Our study provides novel monitoring data for non-antimicrobial PAC abundance and the associated potential ecological risk related to hospitals and urban surface waters in Sri Lanka and further offers valuable information on pre–COVID-19 era PAC distribution in the country. *Environ Toxicol Chem* 2021;00:1–14. © 2021 SETAC

Keywords: Pharmaceutically active compounds; Emerging contaminant; Hospital wastewater; Effluents; Environmental risk assessment; Sri Lanka

INTRODUCTION

Pharmaceutically active compounds (PACs), which include antibiotics, non-antibiotics, and biocides/preservatives (often referred as personal care products), are of great environmental concern owing to their persistence, bioavailability, and potential health risks to aquatic ecosystems (de Solla et al., 2016; Guruge et al., 2019; Segura et al., 2015; Tamura et al., 2017; Verlicchi et al., 2012; Verlicchi & Zambello, 2015). The PACs are introduced to the aquatic environment mainly via direct discharge of raw or treated wastewater from municipal wastewater treatment plants (WWTPs; Al Aukidy et al., 2012; Verlicchi et al., 2012), hospitals (Rodriguez-Mozaz et al., 2015; Verlicchi et al., 2010), industrial WWTPs (Fick et al., 2009), sewage overflow (Launay et al., 2016; Wolf et al., 2012), and agricultural runoff from manure waste treatment facilities (Al Aukidy et al., 2012; Van Epps & Blaney, 2016). The removal efficiencies for PACs vary by treatment method in WWTPs, and occasionally existing treatment facilities are incapable of removing PACs from the influent wastewater before it is released to the receiving water bodies (reviewed by Patel et al., 2019). Thus,

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WWTPs are considered to be one of the major sources of PACs in the aquatic environment, and large variations in PAC concentrations have been observed in different geographic regions (reviewed by Tran et al., 2018). Furthermore, the universal occurrence of a wide range of these substances across various environmental settings poses serious concern to environmental managers and policymakers (Kümmerer, 2009; Patel et al., 2019).

As a rapidly developing nation, Sri Lanka faces increasing demands for a wide range of medications, including nonprescription drugs sold over the counter and via the Internet (Subashini & Udayanga, 2020). The environmental occurrence of pharmaceutical residues is significantly influenced by their consumption pattern (reviewed by Patel et al., 2019). In most cases, urban WWTPs are not widely established in the major cities, and treated wastewaters from healthcare facilities are directly released to neighboring waterways in Sri Lanka. Earlier, we investigated 72 PACs, among which 41 were detected in surface waters across Sri Lanka, and predicted that some of their levels in urban wastewater canals may be high enough to cause serious ecological damage due to hospital wastewater discharge (Guruge et al., 2019). Consequently, another study was conducted to unravel the occurrence of antimicrobials and their association with selection of antimicrobial resistance (AMR) in *Escherichia coli* in wastewater collected from three hospitals and nearby surface waters in the city of Kandy, Sri Lanka (Guruge et al., 2021). That study indicated that hospital effluents had a considerable influence on antimicrobial contamination and AMR downstream. Nevertheless, there is still a large knowledge gap regarding the discharge levels of non-antimicrobial PACs in wastewater from hospitals and their impact on the urban aquatic environment in Sri Lanka. In particular, pre–COVID-19 discharge levels of PACs in hospitals in Sri Lanka have not been well studied. Therefore, the present study was carried out using samples collected in 2016–2018 for antimicrobial and AMR analysis to further investigate the following, which are as yet unknown in Sri Lanka: (1) the occurrence of 45 non-antimicrobial PACs in hospital wastewaters and adjacent surface waters, (2) their removal efficiency, and (3) the associated ecological risk to the most sensitive aquatic species.

MATERIALS AND METHODS

Sample collection

Treated and untreated hospital wastewater samples were collected from three healthcare facilities from the city of Kandy in Sri Lanka. In addition, surface water samples were collected from the upstream Kandy Lake and the downstream Mid Canal waters (Guruge et al., 2021). Mid Canal is one of the longest urban stormwater drainage systems in Sri Lanka; it originates from the Kandy Lake and runs through the city of Kandy before reaching the Mahaweli River. The length of this canal is nearly 6 km, and the depth and width are up to 5 and 15 m, respectively. Throughout its course, this canal receives polluted gray water from adjacent urban and suburban areas that include domestic, industrial, municipal, and medical effluents. The Mid Canal discharges nearly 20,000 m^3/day of wastewater to the Mahaweli River, which covers nearly 16% (10,327 km^2) of Sri Lanka's land cover (Wickramasinghe et al., 2018). Details on the study area, facilities, and samples are given in the Supporting Information, Figure S1 and Table S1. Among the facilities selected, Hospital 1 (H1) is the largest, and treated approximately 190,000 in-hospital patients, followed by Hospital 2 (H2) and Hospital 3 (H3), which served 73,000 and 73,000 patients, respectively, in 2017. Grab water samples were collected in 500-ml clean polypropylene bottles on three occasions in December 2016, September 2017, and September 2018. In general, sampling months represent relatively less rainfall at the sampling area. A total of 34 samples, which included several wastewater samples, were collected in the morning and afternoon from the inlet and outlet of the treatment plants at each facility (Supporting Information, Table S1). All samples were transported to Japan within 48 h after sampling. The samples were kept at −20 °C until chemical residue analysis. The sample quality was adequate to conduct chemical analysis (Guruge et al., 2021).

Analyses of PACs

We analyzed 45 non-antimicrobial PACs, which included six metabolites, and we used 37 isotopic internal standards to maintain the maximum analytical quality of the analytical method (Guruge et al., 2019).

The target compounds in the present study included four nonsteroidal anti-inflammatory drugs (NSAIDs; diclofenac [DIC], indomethacin [IND], mefenamic acid [MEF], and ibuprofen [IBU]), six antihyperlipidemic agents (bezafibrate [BEZ], fenofibric acid [FEN; fenofibrate metabolite], clofibric acid [CLO; clofibrate metabolite], gemfibrozil [GEM], atorvastatin [ATO], and pravastatin [PRA]), five antihypertensive agents (diltiazem [DIL], propranolol [PRO], carvedilol [CAR], losartan [LOS], and amlodipine [AML]), three antihistamic agents (diphenhydramine [DIP], chlorpheniramine [CHLR], and cetirizine [CET]), two antiepileptic agents (carbamazepine [CBZ] and phenytoin [PHE]), an opioid analgesic agent, and two metabolites (tramadol [TRA], *o*-desmethyl tramadol [O-DTRA], and *n*-desmethyl tramadol [N-DTRA]), 10 antidepressant/antipsychotic agents and metabolites (sertraline [SER], norsertaline [NSER; metabolite of sertraline], fluoxetine [FLX], norfluoxetine [NFLX; metabolite of fluoxetine], paroxetine [PAR], fluvoxamine [FLUV], haloperidol [HAL], risperidone [RIS], quetiapine [QUE], and chlorpromazine [CHLM]), eight anxiolytic agents (nitrazepam [NIT], clonazepam [CLON], oxazepam [OXA], flunitrazepam [FLUN], lorazepam [LOR], alprazolam [ALP], etizolam [ETI], and diazepam [DIA]), and five others, including an anti-ulcer agent (rebamipide; REB), an anticoagulant agent (warfarin; WAR), an anti-itch agent (crotamiton; CRO), and a hypnotic agent (zolpidem; SOL; Supporting Information, Table S2). The selected isotopic internal standards are given in the Supporting Information, Table S2.

High-purity (greater than 95%) analytical standards of target PACs, internal standards, and analytical grade organic solvents

including methanol and methyl tert-butyl ether (MTBE) were purchased from commercial suppliers (Guruge et al., 2019). High-purity deionized water was obtained from a Millipore Milli-Q system.

Sample extraction

Samples were extracted according to a previously established method (Guruge et al., 2019; Tanoue et al., 2015). In brief, all the samples were filtered through glass-fiber filters to remove suspended solids. The filtrate (50 ml for surface waters or 5 ml for hospital wastewater) was spiked with internal standards (Supporting Information, Table S2) and then loaded onto an Oasis HLB Plus Light cartridge (30 mg; Waters) preconditioned with MTBE, followed by methanol and Milli-Q water. The cartridge was washed with Milli-Q water and then vacuum-dried. The analytes retained in the cartridge were eluted with methanol/MTBE, and the eluate was concentrated under N_2 flow. The residue was reconstituted in methanol/Milli-Q water (1 ml) and filtered through a cellulose membrane syringe filter. Final solutions were further diluted as necessary.

Instrumental analysis

Identification and quantification of the analytes were performed on an ultra-fast liquid chromatograph system (Shimadzu) coupled to an AB Sciex Qtrap 5500 mass spectrometer (Applied Biosystems Sciex) operating in electrospray ionization positive and negative modes with multiple reaction monitoring (MRM). The chromatographic separation was achieved with an Asentis Express C18 analytical column (Supelco). Detailed information on liquid chromatography (LC) and mass spectrometry (MS) parameters, chromatographic separation, ion source parameters, MRM transitions, and data acquisition were described previously (Guruge et al., 2019).

Quality assurance and quality control

Target compound concentrations were determined by an isotope-dilution method (Guruge et al., 2019). Calibration curves with at least seven points were prepared by plotting the concentration-dependent response factors and the concentrations of the native standards divided by the concentrations of the internal standards. Labeled internal standards were added at a fixed concentration to all calibration solutions (0.005–5 ng/ml). One procedural blank was run for each batch of 12 samples to check for contamination. Accuracy and precision were determined by triplicate analyses of a wastewater sample spiked with native standards at concentrations of 4, 20, and 100 ng/L (Supporting Information, Table S2). Relative recoveries of most target compounds ranged from 70 to 120%, with relative standard deviations of less than 15%, except for PRA (130 ± 13%), CET (130 ± 11%), and LOR (130 ± 13%) at the lowest spiked concentration (i.e., 4 ng/L). Method detection limits (MDLs) were calculated from the standard deviation of seven replicate injections of surface water extracts spiked with native standards at low concentrations, in which each standard gave a peak with a signal-to-noise ratio of approximately 10. In the case of target compounds that are frequently detected in blank procedural samples, MDLs were calculated from the standard deviation of seven replicates of the method blank. The MDL of the target PACs ranged from 0.10 (REB) to 22 (CRO) ng/L for surface water and from 0.41 (REB) to 22 (IBU and CRO) ng/L for hospital wastewater, respectively (Supporting Information, Table S2). The instrumental detection limits were previously reported in Guruge et al. (2019). During the data calculations and statistical analysis, samples with concentrations below the MDL results were considered to be zero.

Removal efficiency of PACs in hospitals

Removal efficiency of PACs in each facility was estimated as the relative removal efficiency (%), which was calculated using the following equation.

$$\text{Removal efficiency (\%)} = \frac{(\text{conc. in influent} - \text{conc. in effluent})}{\text{conc. in effluent}} \times 100 \quad (1)$$

The concentration of PACs was obtained from the analytical data for the filtered water samples. Removal rates were calculated when concentration (conc.) of PACs in influents was greater than the MDL, and zero was assigned when concentration of PAC at effluents was less than the MDL.

Ecological risk estimation

To evaluate the adverse ecological effects of PACs on the aquatic ecosystem, the environmental risks posed by the target compounds to the most sensitive aquatic species were assessed based on risk quotients (RQs). The RQ was calculated from the predicted environmental concentration (PEC) and the predicted no-effect concentration (PNEC; Ministry of the Environment, Government of Japan, 2018; Straub et al., 2019) using Equations (2) and (3). In some studies, the PEC was calculated by applying a dilution factor that was estimated by freshwater availability and domestic sewage discharge (Keller et al., 2014). Because reliable data are not available for Sri Lanka, a 10-fold factor of the mean of measured environmental concentration (MEC_{mean}) was designated as the PEC to estimate the RQ in the present study. A 10-fold dilution ratio is set in some countries for certain pollutants in the effluents to maintain environmental quality standards (Hashimoto, 2020; Kim et al., 2010).

$$RQ = \frac{PEC}{PNEC} \quad (2)$$

$$PEC = \frac{MEC_{mean}}{10} \quad (3)$$

The PNEC was obtained from the available literature, in which PNEC values were calculated from the chronic/subchronic

standard ecotoxicity endpoints in algae, crustaceans, and fish. (The PNEC data we used were listed in Ågerstrand and Rudén [2010], Guruge et al. [2019], and Orias and Perrodin, [2013].) When chronic/subchronic no-observed-effect concentration (NOEC) values were not available, experimental estimations of alternative PNECs were extracted from the literature (Ministry of the Environment, Government of Japan, 2017; Yamamoto et al., 2007). Risk was classified as high (RQ > 1), medium (0.1 < RQ < 1), and low (RQ < 0.1; Verlicchi et al., 2012).

Statistical analysis

The data were first tested for normality and homogeneity of variance. A one-way analysis of vaiance was conducted to test the difference in PAC concentrations among various surface and wastewater samples, applying a $p < 0.05$ significance level (SPSS, Ver 18). Box and whisker plots were applied to present removal efficiencies. Cluster analysis and heat map visualization were performed with $\log_{10}(x+1)$-transformed concentration data. Clustering was carried out on the normalized data using Ward's methods, with squared Euclidean distances as a measure of similarity. For data visualizations, OriginPro (Ver 8.5) and R software (Ver 4.0.3. R Development Core Team, 2020) were used.

RESULTS

Occurrence of PACs in wastewater and surface waters

Among the 45 investigated PACs, 36 were detected in the water samples (Figure 1 and Supporting Information, Table S3). Compounds that were not detected in any samples are not discussed. Among the 36 detected compounds, BEZ (two occasions, at H1 effluent and Mid Canal), ZOL (two occasions, in H2 and H3 influent), NSER (two occasions, in H1 and H3 influent), and CLON (three occasions, two in H1 influents and one in H2 effluent) were detected at low frequencies. A summary of the concentrations (min–max, mean) and detection frequencies of the 32 remaining compounds are presented in Table 1. There were six compounds (MEF, GEM, LOS, CET, CBZ, and PHE) remaining in all samples with a 100% detection frequency.

All NSAIDs were detected at high concentrations in the influent raw wastewaters. In the hospital influent waters, DIC, MEF, and IBU were detected at up to 8040, 12,120, and 7280 ng/L; however, the IND concentration (up to 252 ng/L) was 1 order of magnitude lower than that of the other NSAID compounds. The DIC level detected in influent of H1 was significantly greater than that of H3 ($p < 0.05$). The signatures of all NSAIDs were also pervasive in the downstream waste canal, where MEF was detected at up to 2520 ng/L. The total mean concentrations of NSAIDs in hospital effluents were 7365, 5557, and 1283 ng/L for H1, H2, and H3, respectively. The concentration was 1085 ng/L in Mid Canal water, an increase of 3 orders of magnitude over the concentration found in Kandy Lake.

Among lipid regulatory drugs, a high concentration of FEN was detected at up to 2700 ng/L in the influent, whereas GEM and ATO were detected at up to 340 and 14,620 ng/L in the effluents. In all samples collected, GEM was detected, and its mean concentration in the effluent waters of H1 was significantly ($p < 0.001$) higher than that in its influent and in all sample sites from other hospitals and surface waters. In addition, ATO was detected at a significantly higher concentration in the effluent waters of H1 compared with its influent ($p < 0.01$), both influents and effluents of H2 and H3 ($p < 0.001$), and Mid Canal. The total mean concentrations of these three lipid regulatory drugs in hospital effluents were 9509, 868, and 486 ng/L for H1, H2, and H3, respectively. The concentration was 209 ng/L in Mid Canal water, an increase of

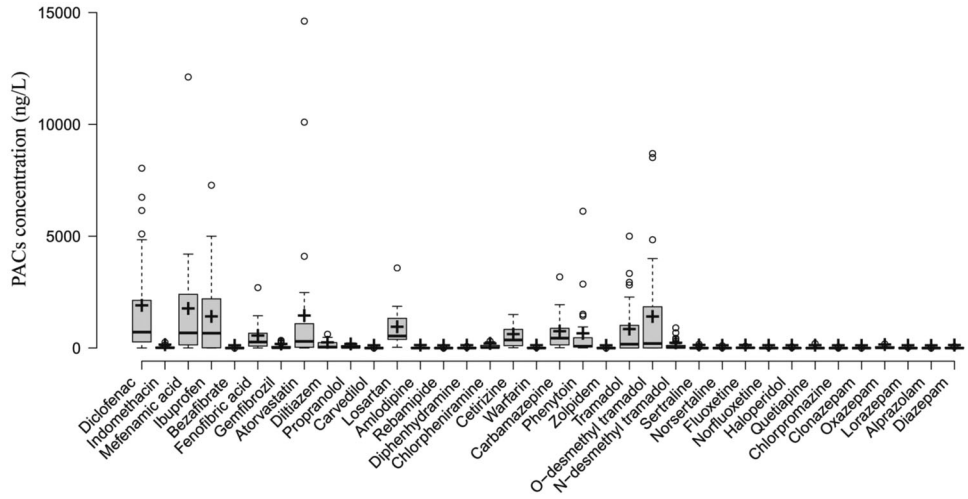

FIGURE 1: Box and whiskers plot for overall measured concentrations of pharmaceutically active compounds (PACs) in surface and hospital wastewater treatment plants (WWTPs) wastewaters in Sri Lanka. The center lines and plus sign (+) represent the median and mean values, respectively; box limits indicate the 25th and 75th percentiles; whiskers extend 1.5 times the interquartile range from the 25th and 75th percentiles, and outliers are represented by open circles.

TABLE 1: Concentrations (ng/L) of pharmaceutically active compounds (PACs) measured in Kandy Lake, hospital influents, hospital effluents, and the Mid Canal

			Hospital 1				Hospital 2				Hospital 3					
	Kandy Lake		Influent		Effluent		Influent		Effluent		Influent		Effluent		Mid Canal	
Compound	Mean range	Det. %	Mean range	Det. %	Mean range	Det. %	Mean range	Det. %	Mean range	Det. %	Mean range	Det. %	Mean range	Det. %	Mean range	Det. %
DIC	—		4016		3209		3153		2070		470		273		391	
	<MDL–	0	1419–6740	100	1881–6150	100	586–8040	100	7.9–3680	100	206–835	100	12–435	100	272–483	100
IND	—		55		82		58		19		13		3.7		5.9	
	<MDL	0	10–134	100	66–95	100	<MDL–252	80	<MDL–52	60	<MLD–46	40	<MDL–19	20	<MDL–13	67
MEF	4.0		3614		2608		2276		2261		900		355		387	
	3.7–4.4	100	11–12 120	100	3.3–4000	100	990–4200	100	37–4020	100	326–2520	100	40–597	100	138–552	100
IBU	—		1440		1467		1427		1206		3007		650		301	
	<MDL	0	161–5000	100	<MDL–2200	100	<MDL–3160	80	211–2230	100	<MDL–7280	80	<MDL–2220	60	71–438	100
FEN	—		734		608		134		394		887		436		39	
	<MDL	0	40–1442	100	526–662	100	20–272	100	124–845	100	127–2700	100	145–880	100	9.9–88	100
GEM	4.7		17		300		47		120		3.2		11		37	
	4.2–5.1	100	6.4–46	100	314–340	100	2.1–124	100	5.9–218	100	0.79–7.7	100	4.2–20	100	24–48	100
ATO	—		2176		8602		745		354		497		39		133	
	<MDL	0	934–4100	100	1085–14 620	100	32–1580	100	<MDL–550	80	24–2040	100	<MDL–43	80	17–216	100
DIL	—		390		447		98		117		21		0.93		14	
	<MDL	0	360–412	100	251–616	100	39–228	100	<MDL–242	80	<MDL–88	60	<MDL–4.7	20	7.6–22	100
PRO	—		146		184		25		61		45		23		13	
	<MDL	0	97–222	100	155–227	100	22–29	100	<MDL–109	80	17–132	100	<MDL–42	80	9.9–19	100
CAR	—		4.5		7.4				—		—		—		—	
	<MDL	0	<MDL–15	60	3.5–15	100	<MDL	0	<MDL	0	<MDL	0	<MDL	0	<MDL	0
LOS	37		1628		1527		991		849		549		382		511	
	33–44	100	388–3580	100	940–1868	100	547–1474	100	454–1432	100	191–1006	100	214–530	100	186–730	100
AML	—		29		24		17		8.9		4.6					
	<MDL	0	14–42	100	21–29	100	<MDL–50	40	<MDL–14	80	<MDL–23	20	<MDL	0	<MDL	0
REB	0.25		—		—		—		—		—		0.09		13.8	
	0.20–0.28	100	<MDL	0	<MDL	0	<MDL	0	<MDL	0	<MDL	0	<MDL–0.47	20	0.55–39	100
DIP	—		5.9		22		16		6.4		6		5.47		3.0	
	<MDL	0	<MDL–12	80	18–32	100	<MDL–69	60	<MDL–17	80	<MDL–12	60	<MDL–15	80	2.2–3.9	100
CHLR	—		149		262		79		52		103		38		11	
	<MDL	0	12–273	100	216–307	100	5.5–193	100	<MDL–111	80	5.8–282	100	<MDL–110	80	6.5–14	100
CET	15		628		1042		674		499		478		416		136	
	14–16	100	71–1498	100	802–1420	100	34–1214	100	130–956	100	86–1216	100	163–834	100	85–200	100
WAR	0.14		18		33		6.0		5.9		1.2		0.56		1.9	
	<MDL–0.41	33	9.7–39	100	24–41	100	3.0–9.8	100	<MDL–11	80	<MDL–3.5	60	<MDL–1.8	40	1.0–2.8	100
CBZ	18		1337		1500		598		650		486		214		172	
	15–21	100	498–3180	100	875–1940	100	224–990	100	387–956	100	73–1682	100	126–368	100	93–234	100
PHE	6.6		2248		1487		107		254		34		60		78	
	5.8–7.1	100	490–6120	100	1436–1516	100	62–190	100	60–460	100	7.8–71	100	19–104	100	43–113	100
TRA	2.8		1950		3032		484		609		—		2.3		130	
	2.1–3.9	100	564–5000	100	2820–3335	100	<MDL–1076	80	3.6–1222	100	<MDL	0	<MDL–8.3	40	39–208	100
O-DTRA	0.25		3278		5847		825		1056		8.7		8.5		155	
	<MDL–0.75	33	1002–8520	100	4000–8700	100	4.1–1844	100	3.5–2060	100	<MDL–24	80	<MDL–12	80	56–260	100
N-DTRA	1.1		327		507		49		72		—		2.6		22	

(*Continued*)

TABLE 1: (Continued)

	Kandy Lake		Hospital 1				Hospital 2				Hospital 3				Mid Canal	
			Influent		Effluent		Influent		Effluent		Influent		Effluent			
Compound	Mean range	Det. %	Mean range	Det. %	Mean range	Det. %	Mean range	Det. %	Mean range	Det. %	Mean range	Det. %	Mean range	Det. %	Mean range	Det. %
	0.92–1.2	100	77–902	100	406–680	100	<MDL–94	80	3.4–120	100	<MDL	0	<MDL–5.2	80	6.2–29	100
SER	—		8.8		19		17.2		55		5.7		7.3			
	<MDL	0	<MDL–37	40	11–32	100	<MDL–86	20	<MDL–169	80	<MDL–28	20	<MDL–32	40	<MDL	0
FLX	—		25		29		21		31		7.2		2.5			
	<MDL	0	12–36	100	22–42	100	<MDL–42	60	<MDL–80	80	<MDL–28	40	<MDL–12	20	<MDL	0
NFLX	—		9.8		11.4		8.1		11		—					
	<MDL	0	<MDL–18	60	<MDL–19	67	<MDL–28	40	<MDL–44	40	<MDL	0	<MDL	0	<MDL	0
HAL	—		5.1		9.4		17		23		0.5					
	<MDL	0	2.8–9.1	100	7.4–13	100	<MDL–59	80	<MDL–59	80	<MDL–2.6	20	<MDL	0	<MDL	0
QUE	—		1.6		7.8		41		4.8		3.0		1.4			
	<MDL	0	<MDL–8.1	20	6.1–8.9	100	<MDL–200	40	<MDL–13	40	<MDL–15	20	<MDL–7.1	20	<MDL	0
CHLM	—		7.0						1.16		15					
	<MDL	0	<MDL–21	60	<MDL	0	<MDL	0	<MDL–5.8	20	<MDL–50	40	<MDL	0	<MDL	0
OXA	—		89		83.5		54		44		3.5		6.6		4.3	
	<MDL	0	26–183	100	70–106	100	<MDL–130	80	<MDL–63	80	<MDL–9.8	40	<MDL–6.5	80	<MDL–7.3	67
LOR	—		5.1				9.4		14		2.2					
	<MDL	0	<MDL–13.4	20	<MDL	0	<MDL–20	60	<MDL–29	80	<MDL–11	20	<MDL	0	<MDL	0
ALP	—		3.6		3.5		0.25		0.41		—					
	<MDL	0	<MDL–11	60	1.8–4.4	100	<MDL–1.3	20	<MDL–1.2	40	<MDL	0	<MDL	0	<MDL	0
DIA	—		33		47		14		17		—		1.17		2.9	
	<MDL	0	24–48	100	41–63	100	<MDL–35	80	<MDL–30	80	<MDL	0	<MDL–3.1	40	2.4–3.6	100

ALP = alprazolam; AML = amlodipine; ATO = a torvastatin; CAR = carvedilol; CBZ = carbamazepine; CET = cetirizine; CHLM = chlorpromazine; CHLR = chlorpheniramine; Det. (%) = detection frequency; DIA = diazepam; DIC = diclofenac; DIL = diltiazem; DIP = diphenhydramine; FEN = fenofibric acid; FLX = fluoxetine; GEM = gemfibrozil; HAL = haloperidol; IBU = ibuprofen; IND = indomethacin; LOR = lorazepam; LOS = losartan; MDL = method detection limit; MEF = mefenamic acid; N-DTRA = n-desmethyl tramadol; NFLX = norfluoxetine; O-DTRA = o-desmethyl tramadol; OXA = oxazepam; PHE = phenytoin; PRO = propranolol; QUE = quetiapine; REB = rebamipide; SER = sertraline; TRA = tramadol; WAR = warfarin.

2 orders of magnitude compared with its concentration in Kandy Lake.

Among antihypertensive drugs, LOS was detected in all the samples, and its concentration in H1 influent water (up to 3580 ng/L) was significantly higher ($p < 0.05$) than that in Kandy Lake water (33.4–43.8 ng/L). Other members of this group, such as DIL, PRO, and CAR, were detected at levels several-fold lower than those of LOS. Nevertheless, the DIL and PRO levels found in H1 wastewaters were significantly greater than those in H1 and H2 and Mid Canal ($p < 0.05$–0.001). The total mean concentrations of these three compounds in hospital effluents were 2190, 1036, and 406 ng/L for H1, H2, and H3, respectively. The concentration was 538 ng/L in Mid Canal water, an increase of 14-fold compared with the concentration in Kandy Lake.

Among the three antihistamine drugs detected in all samples, CET was predominant, with the highest level of 1498 ng/L found in H1 influents. On the other hand, the highest concentrations for DIP and CHLR were 32 and 307 ng/L, respectively, which were detected in H1 effluents. The CHLR levels in the H1 effluents were significantly higher than those found in H2 and H3 ($p < 0.05$). The total mean concentrations of these three compounds in hospital effluents were 1326, 557, and 460 ng/L for H1, H2, and H3, respectively. The concentration was 151 ng/L in Mid Canal water, an increase of 10-fold compared with the concentration in Kandy Lake.

Among the antiepileptic drugs, CBZ and PHE were detected in all the samples;concentrations ranged from 15.3 to 3180 and 5.8 to 6120 ng/L, respectively. The concentration of PHE in the influent water of H1 was significantly higher ($p < 0.05$) than that at the other two hospitals. The total mean concentrations of these two compounds in hospital effluents were 2988, 904, and 274 ng/L for H1, H2, and H3, respectively. The concentration was 250 ng/L in Mid Canal water, which was 1 order of magnitude higher than the concentration in Kandy Lake.

The highest concentrations for the opioid analgesic TRA and its active metabolites O-DTRA and N-DTRA were found to be 5000, 8700, and 902 ng/L, respectively,and the latter was dominant in the effluents. All three compounds were detected at 100% in Mid Canal water. These compounds in H1 effluents were significantly greater ($p < 0.01$) than those found in both influents/effluents at H2 and H3, Kandy Lake, and Mid Canal. The total mean concentrations of these three compounds in hospital effluents were 9386, 1737, and 13 ng/L for H1, H2, and H3, respectively. The concentration was 308 ng/L in Mid Canal, an increase of 2 orders of magnitude over that in Kandy Lake.

Among the 10 detected psychotropic agents, the highest concentrations (in ng/L) for SER, FLX, NFLX, HAL, QUE, CHLM, OXA, LOR, ALP, and DIA were found to be 169 (H2), 80 (H2), 44 (H2), 59 (H2), 200 (H2), 21 (H1), 183 (H1) 29 (H2), 11 (H1), and 63 ng/L (H1), respectively. Only the OXA concentration in H1 influent was significantly higher than that in the H3 influent ($p < 0.05$). However, the same concentrations were not detected at Kandy Lake, and only OXA and DIA were found at the Mid Canal site. Furthermore, DIA levels in H1 effluent were significantly higher than those in H2 and H3 and Mid Canal ($p < 0.01$).

Among the PACs we detected, the maximum concentration of WAR (41 ng/L), an anticoagulant agent, was detected in H1 effluents. Its mean concentration was significantly greater in H1 influents than in either influents or effluents at H1 and H2 ($p < 0.05$). In contrast, REB, an anti-ulcer agent, was detected frequently (5/6 occasions) in the surface waters, with higher concentrations detected in the Mid Canal water (39 ng/L).

Overall, the total numbers of PACs detected in Kandy Lake, H1, H2, H3, and Mid Canal were 11, 33, 32, 32, and 23,

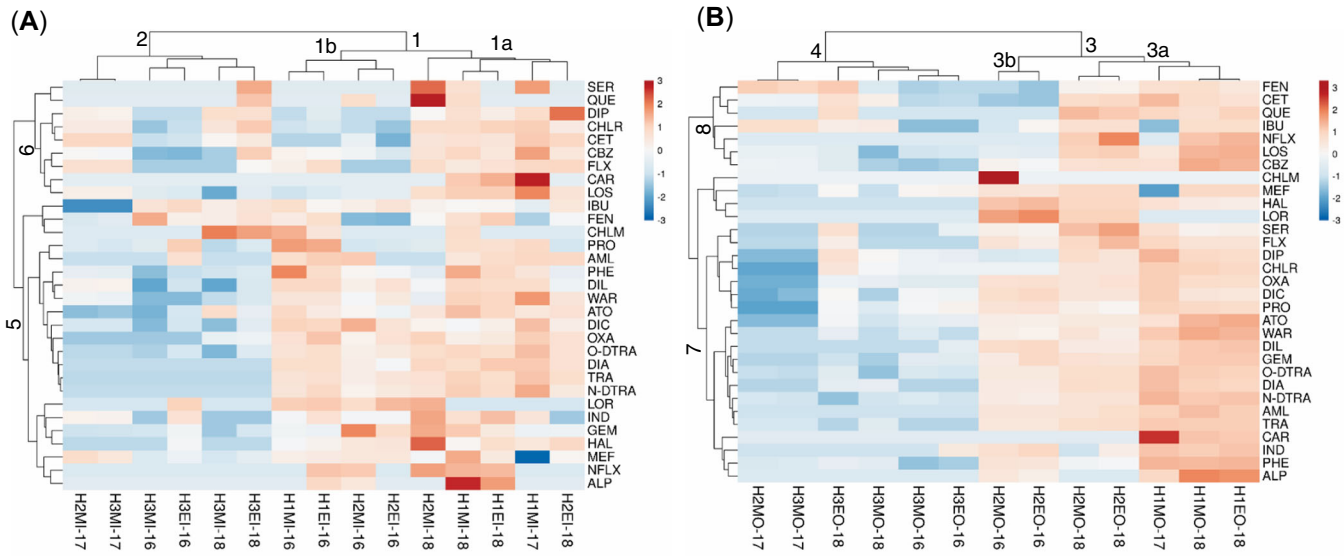

FIGURE 2: Heat map and cluster analysis of various pharmaceutically active compounds (PACs) detected during the 3-year sampling campaign in hospital wastewater treatment plants (WWTPs) from Sri Lanka: (**A**) influent and (**B**) effluent. The color of each cell represents the detected concentrations (ng/L) in different samples (diurnal, spatial, and interannual variations). Concentrations of individual compounds were normalized (by row). The color gradient represents the relative concentration from the lowest (–3) to highest (3). Sample labels are as follows: H = hospital; numbers 1, 2, and 3 = hospital number; M = morning; E = evening; I = influent water; O = effluent water, all followed by the numbers 16, 17, and 18 representing years 2016, 2017, and 2018, respectively. For PAC abbreviations, see footnote to Table 1.

respectively. The measured total mean concentrations of PACs in wastewater from hospitals were 24,398, 11,990, and 7555 ng/L for influents and 33,007, 10,867, and 2943 ng/L for effluents in H1, H2, and H3, respectively. The mean PAC concentrations in Kandy Lake and Mid Canal water were 90 and 2563 ng/L, respectively.

Heat map–hierarchical cluster analysis in hospital influent and effluent waters

The concentration heat map (Figure 2) shows the diurnal, interhospital, and interannual variability of target PACs. In the influent and effluent waters, the higher variation in concentrations (red color) of individual drugs was predominantly located in H1 and H2.

The dendrograms for the different influent samples (horizontal axis) of the hospitals and time scales revealed two major clusters (Figure 2A). The first cluster was divided into two subclusters (1a and 1b). The first subcluster (1a) was formed by influent samples of H1 and H2 collected in 2017 and 2018. The second subcluster (1b) was formed by influent samples of H1 and H2 collected in 2016. The second cluster was formed predominantly by influent samples of H3 for all 3 years. The clustering of effluent samples also revealed two major clusters similar to the influent samples (Figure 2B). The first cluster was subdivided into two clusters (3a and 3b). The first subcluster (3a) was formed with effluent samples of H1 and H2 collected in 2017 and 2018. The second subcluster (3b) of cluster 1 was grouped by effluent samples of H2 during 2016. Similar to the influent samples, the second cluster of effluent samples was again formed by the effluent samples of H3 during all 3 years. These results clearly indicate that the drug usage in Hospital 3 is quite different than that in the other two hospitals.

Clustering of 31 PACs (vertical axis, Figure 2), on the other hand, revealed more complicated patterns in both influent and effluent waters. In influent samples, a distinct cluster of nearly 22 (1A cluster 5) of 31 detected analytes was observed. However, the next group (nine compounds, 1A cluster 6) was comprised of antihistamic drugs such as DIP, CHLR, and CET, antidepressant drugs such as FLX, two antihypertensive agents, CAR and LOS, antiepileptic agents such as CBZ, and antipsychotic agents such as QUE. In the effluent, the first distinct cluster was formed with 24 of 31 compounds (1B cluster 7), whereas the remaining seven PACs (FEN, CET, QUE, IBU, NFLX, LOS, and CBZ; 1B cluster 8) formed the second cluster.

Removal of PACs in the aquatic phase

The summarized data for removal rates of 33 target compounds at the three hospitals are given in Figure 3, and the data for individual hospitals are given in the Supporting Information, Figure S2. The estimated removal efficiencies varied extensively,

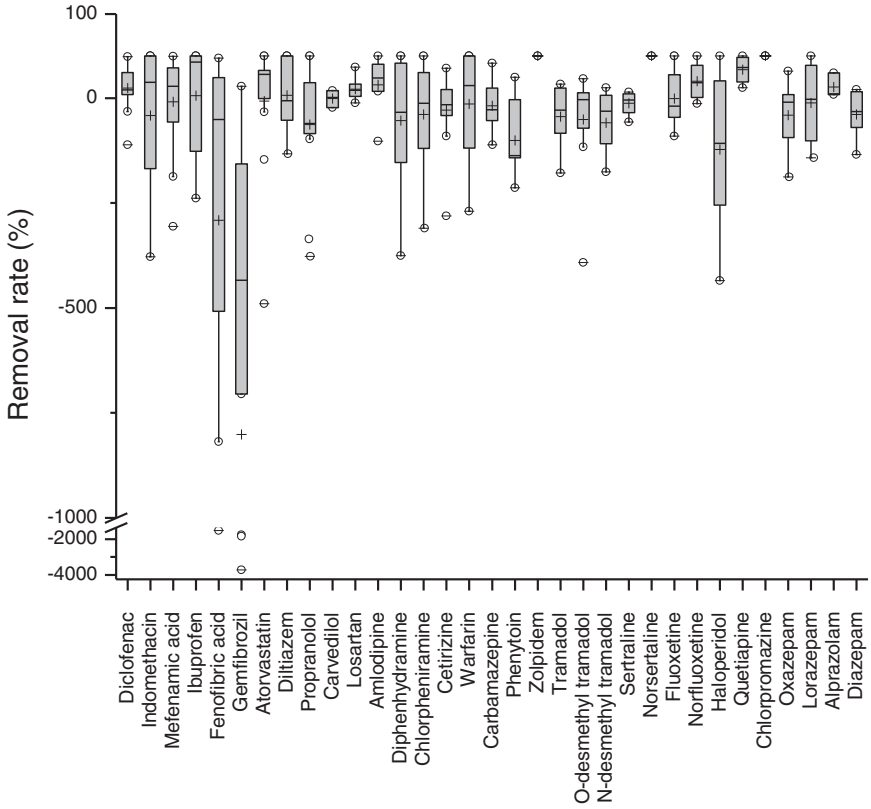

FIGURE 3: Box and whiskers plot for removal efficiencies (%) of pharmaceutically active compounds (PACs) in hospital wastewater treatment plants (WWTPs) during the present study. The center lines and plus sign (+) represent the median and mean values, respectively; box limits indicate the 25th and 75th percentiles; whiskers extend 1.5 times the interquartile range from the 25th and 75th percentiles, and outliers are represented by open circles.

from negative to 100%, for most of the PACs in our study. Only a few compounds (ZOL, NSER, QUE, CHLM, and ALP) showed positive removal in the wastewater, reaching 100%. In addition, on several occasions large negative removal rates were observed for FEN and GEM; we found an increasing trend of these compounds in effluents after treatment. Compounds IBU, DIL, AML, ZOL, NSER, HAL, and LOR showed more than 79% mean removal efficiencies in H3. Nevertheless, depending on the compound, a large disparity in removal rates was found among hospitals (Supporting Information, Figure S2).

Ecological risk assessment

The MECs of compounds detected in water samples and the PNECs of the most sensitive aquatic species were selected to estimate the RQs. The PNEC data were available for 28 compounds detected in water samples; however, the estimated RQs revealed that only 14 of them pose low to high risk to various aquatic organisms (Figure 4 and Supporting Information, Table S4). The ecological risk of PACs detected in Kandy Lake was insignificant. The highest risk (RQ: 3.01–30.07) to fish in all hospital wastewaters and Mid Canal water was posed by IBU. Likewise, the second highest mean RQs were observed for CHLM in the influent waters of H1 and H2, and effluents of H2, with a range of 1.32 and 17.55. High risk in H1 influent and effluent waters was found for TRA, with RQs ranging between 1.3 and 3.03. The RQ values estimated for TRA for wastewaters of H1 were nearly 3- to 10-fold higher than in the H2 wastewater or Mid Canal water downstream. Among other measured drugs, DIC, MEF, and PRO posed a medium risk either to fish or crustaceans in the H1 and H2 wastewaters. In addition, ATO and LOS revealed moderate to low risk to crustaceans in H1 wastewater. It is noteworthy that only seven PACs were found to show low to high risk to aquatic organisms in the Mid Canal water.

DISCUSSION

Occurrences of PACs in hospital wastewaters and adjacent surface waters

The present study is the first to report the presence of a large number of non-antimicrobial PACs in wastewater in Sri Lanka. The presence of not all, but most of these drugs at WWTPs has been reported in the literature (Azuma et al., 2016; reviewed in Balakrishna et al., 2017; Kleywegt et al., 2016; Kostich et al., 2014; reviewed in Petrie et al., 2015; reviewed in Tran et al., 2018; Supporting Information, Table S5).

Among the NSAIDs, the mean MEF found in H1 and H2 was almost 2-fold higher than that found in Indian WWTPs (reviewed by Balakrishna et al., 2017). The highest DIC concentrations detected in H1 and H2 exceeded the highest levels reported in WWTPs in China (Sun et al., 2016; Y. Y. Yang et al., 2017), Korea (Behera et al., 2011), India (Anumol et al., 2016), Singapore (Tran & Gin, 2017), the United States (X. Yang et al., 2011), Canada (Lee et al., 2005; Metcalfe et al., 2003), the United Kingdom (Kasprzyk-Hordern et al., 2009), Greece (Kosma et al., 2010, 2014), and Austria (Clara, Kreuzinger, et al., 2005; Clara, Strenn, et al., 2005). The IBU levels were within the range reported elsewhere; however, the highest concentration detected at these hospitals was at least 1 order of magnitude lower than that in Japan (Azuma et al., 2016), the United States (Palmer et al., 2008), and Canada (Guerra et al., 2014; Kleywegt et al., 2016).

Among the antihyperlipidemic agents, GEM was detected in all the samples; however, ATO residue levels were found to be higher, especially at H1, which was the largest among the studied healthcare facilities. It is noteworthy that ATO showed the highest concentration for the PACs analyzed in our study, and its mean concentration was 1 order of magnitude higher than the levels reported in WWTPs in India (Balakrishna et al., 2017; Subedi et al., 2017). In addition, GEM concentrations

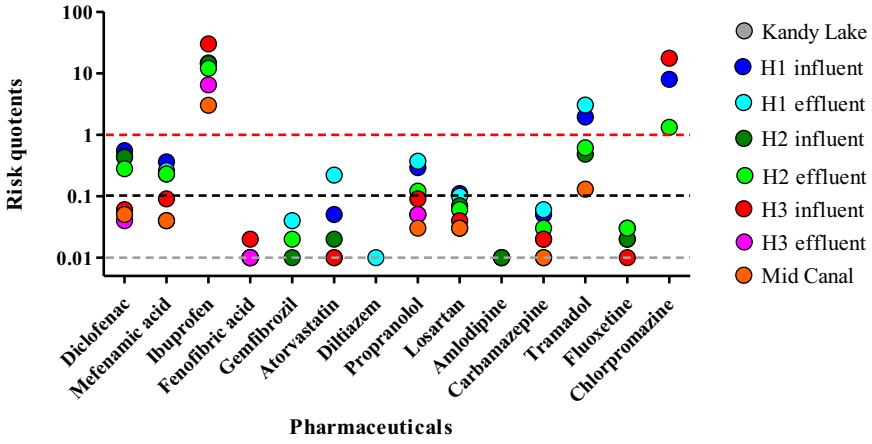

FIGURE 4: Mean risk quotients (RQs) derived using mean concentrations of pharmaceutically active compounds in surface and hospital (H) wastewater treatment plants (WWTPs) wastewaters in Sri Lanka, for the most sensitive aquatic species. The gray, black, and red colored horizontal dotted lines represent low, moderate, and high environmental risks, respectively.

found in hospitals were within the same range as those in Asian countries like China (Sui et al., 2011; Sun et al., 2016) and Korea (Behera et al., 2011); nevertheless, the highest value found in Sri Lanka was at least 1 order of magnitude lower than those reported in Canada (Metcalfe et al., 2003), Spain (Rosal et al., 2010), and Greece (Kosma et al., 2010). The differences in obesity rates between Asian and Western countries could be a possible reason for the relatively lower levels of GEM in the Asian countries.

Among antihypertensive agents, LOS was detected in all samples, suggesting its extensive usage in the medical environment in Sri Lanka. Data on LOS are not well reported; however, the concentrations found in our study were much higher than those reported in Germany (Gurke et al., 2015) and Portugal (Santos et al., 2013). Data for CAR are also previously not well documented. Our data indicated that PRO levels found in the hospital wastewaters were in the range of those reported for China (Sui et al., 2011; Sun et al., 2016), the United States (Balakrishna et al., 2017), Greece (Papageorgiou et al., 2016), and Spain (Rosal et al., 2010). The AML and DIL concentrations were similar to those reported for effluent samples from the United States (Kostich et al., 2014).

Among antihistamic agents, CET was detected in all samples at a similar concentration to that in the influents, suggesting its common usage in these three hospitals. The DIP levels in Sri Lankan hospital wastewater were lower than those in several WWTPs in India (Balakrishna et al., 2017). The CHLR concentration has rarely been reported elsewhere.

The anticoagulant agent WAR was found to be present at low concentrations compared with other tested analytes. The WAR levels detected in our study were several-fold lower than those previously reported in hospital wastewaters in Portugal (Santos et al., 2013). Nevertheless, WAR was detected on all occasions in H1 and Mid Canal waters; WAR was detected in at least 40% of surface water samples in Sri Lanka (Guruge et al., 2019).

The antiepileptic agents CBZ and PHE were detected in all samples, suggesting their wide usage in Sri Lanka. The carbamazepine is considered as a conservative indicator as it detects in effluents at high concentrations (Kasprzyk-Hordern et al., 2009; Y. Y. Yang et al., 2017), and the levels we found were within the range of reported data for India (Subedi et al., 2017), South Korea (Behera et al., 2011), Switzerland (Kahle et al., 2009), and the United Kingdom (Kasprzyk-Hordern et al., 2009), but higher than those noted for China (Sun et al., 2016; Y. Y. Yang et al., 2017) and the United States (Kostich et al., 2014; Mohapatra et al., 2016). The concentration of CBZ reported in the influent waters of hospitals was quite comparable to that in influent waters of WWTPs from Saudi Arabia (Al Qarni et al., 2016). The PHE levels in H1 and H2 were found to be several-fold higher than the levels reported in a WWTP in Queensland, Australia (Cardenas et al., 2016). Occurrence of CBZ and PHE in the surface waters is common, suggesting their ubiquitous usage in Sri Lanka (Guruge et al., 2019).

The opioid analgesic TRA and its active metabolites, O-DTRA and N-DTRA, were predominant in H1, which was the largest of the hospitals. The aggregate of all three compounds at H3 was 2 to 3 orders of magnitude lower than that at the other two hospitals, suggesting that TRA use at H3 was insignificant. However, the highest TRA levels detected in our study were several-fold lower than those reported for wastewaters in the United Kingdom (reviewed by Petrie et al., 2015).

We detected 10 psychotropic agents in wastewater, but only OXA and DIA were found in the Mid Canal water. This drug class was the least frequently detected among all the analytics in the hospitals, suggesting its lower usage, probably due to metabolic degradation and chemical transformation (Subedi & Kannan, 2015). Nevertheless, FLX and NFLX levels were similar to those reported in wastewaters from the United Kingdom (reviewed by Petrie et al., 2015) and Canadian hospitals and care facilities (Kleywegt et al., 2016), but lower than those reported at WWTPs in the United States (Subedi & Kannan, 2015). The concentrations of those two compounds with SER and ALP levels noted in hospitals in our study were comparable to those detected in effluents from 50 WWTPs in the United States (Kostich et al., 2014). The OXA concentrations in H1 and H2 were at least 1 order of magnitude lower than those reported at a WWTP in Australia (Cardenas et al., 2016), a few times higher than in WWTPs in Albany, New York (USA; Subedi & Kannan, 2015), and similar to data reported in the United Kingdom (Petrie et al., 2015). The mean DIA levels found in H1 and H2 were a few orders higher than those reported at WWTPs in the United Kingdom and the United States (Petrie et al., 2015; Subedi & Kannan, 2015). The occurrence of both QUE and LOR in hospital effluents was several-fold lower than those reported in WWTPs in Albany (Subedi & Kannan, 2015). However, HAL and CHLM have rarely been discussed in the literature.

Interestingly, an anti-ulcer agent (REB) was detected predominantly in the canal water, implying that its contamination had arisen through domestic sewage. Even though we could detect a wide range of non-antimicrobial PACs in Kandy Lake and Mid Canal, their concentrations, in general, did not exceed those of reported data from elsewhere (Bu et al., 2013; Ngo et al., 2021; Y. Y. Yang et al., 2018). However, DIC, IBU, TRA, and LOS concentrations in the Mid Canal were higher than those reported in several rivers and other surface waters in Columbia (Aristizabal-Ciro et al., 2017), Portugal (Paíga et al., 2016), Serbia (Petrović et al., 2014), Singapore (You et al., 2015), and Sweden (Lindim et al., 2016). Nevertheless, the mean levels of all the PACs measured at those two sites in the present study were at least a few folds higher than those from data reported from the samples collected in 2013, showing that aquatic pollution has an increasing tendency in the study area (Guruge et al., 2019). The mean total PACs concentration in the Mid Canal water was 28-fold greater than that in Kandy Lake, indicating that hospital effluents have a direct influence on downstream water quality.

The concentration heat map results clearly revealed variable drug usage among the three hospitals. In influent, the highest concentration of target PACs was found at H1, revealing that the corresponding compounds might be administered under different therapeutic conditions in this hospital. Furthermore, the concentration hotspots in H1 and H2 morning samples in

2017 and 2018 are noteworthy, because this could be a one-time incident of direct disposal (Petrie et al., 2016). In the effluent samples, a high load of individual compounds was primarily observed at both H1 and H2, suggesting poor removal efficiencies of target PACs. Heat map data clearly revealed that different PAC compounds were primarily separated based on their spatial (interhospital) and temporal (interday; annual) variations; therefore, comparison among compounds is not appropriate (vertical clusters). This clearly indicates a complex usage pattern for each drug, and a generic trend is difficult to identify among the various drugs investigated (Kosma et al., 2020).

Removal rates of PACs in hospitals

In the present study, removal rates were estimated for 3, 5, and 5 occasions at H1, H2, and H3, respectively, during the 3 years. The data revealed large dissimilarities in removal rates for PACs at all of the hospitals. It has been suggested that removal rates of PACs could potentially differ due to their physiochemical properties and the nature of treatment process employed in treatment plants. A recent review, which summarized the removal data for PACs reported in 22 research articles, noted that removal rates of several PACs comprising NSAIDs, antihypertensive agents, psychoactive agents, and lipid regulators can be wide ranging: from negative or no removal to 100% (Tran et al., 2018). After being metabolized within the human body, pharmaceuticals reach the WWTPs as conjugated, oxidized, or transformed metabolites along with their parent compounds (Testa et al., 2012). However, deconjugation of these transformed metabolites into their parent compounds via enzymatic activity and abiotic processes is widely reported in the sewage or effluents of WWTPs and could be the possible explanation for the observed negative removal in our study (Nguyen et al., 2021). In contrast, GEM seems to be positively removed from the full-scale WWTPs, but it showed the most negative removal rate at H1 and H2. The grab sampling uncertainty might add some variation in PAC levels and might accordingly affect the evaluation of the removal rates (Ort et al., 2010; Tran et al., 2018). Our data also revealed that removal rates of PACs drastically fluctuated with time (morning and evening), suggesting that a single snapshot sample would not provide an accurate depiction of performances of treatment plants and that a long-time monitoring program would be required to accurately predict their treatment efficiencies (Wang & Wang, 2016). Treatment at H1 and H2 operated only with the conventional activated sludge process, which was not designed for removal of most of the PACs. The smallest facility, H3, which had additional rotating biological contactors to treat wastewater, showed better performance in regard to removal rates for most of the PACs analyzed. The high input, limited treatment, and low contact time for treatment could contribute to a lower PAC removal efficiency (Subedi et al., 2017). Furthermore, the increase of PACs in effluents could also be a result of the deconjugation of parent compounds during treatment of the raw wastewater (Ratola et al., 2012).

Wild fish can accumulate IND, MEF, DIP, and HAL (Tanoue et al., 2015), which we observed incrementally in the hospital discharge after treatment. The types and concentrations of PACs accumulated in fish collected near effluent sites were greater than those detected in fish at the less contaminated sites (Muir et al., 2017). Taken together, our results demonstrate that the existing treatment processes utilized by these hospitals may be incapable of removing most of the target compounds, instead, they actually increase PAC levels in the effluents and severely affect the downstream receiving canal and river.

Ecological risk of PACs to aquatic organisms

The IBU levels were several orders greater in the hospital wastewaters and Mid Canal, and chronic exposure can cause a decrease in the survival of Japanese medaka (Han et al., 2010). The concentrations detected for TRA at H1 were found to pose a high risk associated with a delay in fish egg hatching (Sehonova et al., 2016). In addition, estimated RQs indicated that more than a few other compounds, such as CHLM, could pose high ecological risk to crustaceans (Orias & Perrodin, 2013), especially in H1 and H2 wastewater. The NSAID DIC causes increased dysmorphic development in fish (Yokota et al., 2018), PRO can increase larval hatching of fish (Yamamoto et al., 2007), and LOS reduces crustacean reproduction (Ministry of the Environment, Government of Japan, 2018). Previously, using samples collected in 2013, it was reported that only IBU levels in the canal posed a high risk for fish (Guruge et al., 2019). Collectively, the current data show that ecological damage related to IBU, together with several other PACs in Mid Canal waters, remained.

In the present study, the concentrations of 28 PACs found in the hospitals' wastewater and adjacent surface waters posed low to high risk of ecological damage in the aquatic environment. The IBU and CBZ levels detected in treated effluents in Canada exceeded the ecotoxicological criteria by several orders of magnitude (Kleywegt et al., 2016). Moreover, DIC and IBU in surface waters in China (Bu et al., 2013) were identified as the most potent drugs associated with high ecological risk in aquatic systems. Five chemicals in the 2013 survey (Guruge et al., 2019) and 11 chemicals in the present study (2016–2018) were found to pose more than a moderate risk (RQ more than 0.1) to aquatic life in the Mid Canal water. This probably reflects the increasing demand for medications and inadequate treatment of hospital and domestic wastewater before its release into receiving waters. The Kandy Lake is known for its monoculture of fish like tilapia (*Oreochromis mossambicus* and *Oreochromis niloticus*), and the Mid Canal is serves as a common habitat for species such as Korali (*Tilapia mossambica*), and Kavaiya (*Anabas testudineus*; Silva, 2003).

CONCLUSIONS

The present study reports the first data on occurrence, removal, and ecological risk of 45 PACs, belonging to various therapeutic categories, in three healthcare facilities and their

adjacent receiving urban waters over a period of 3 years in Sri Lanka. Total PACs concentrations varied from 83 to 37,562 ng/L. The NSAIDs were the predominant PACs noted in the present study. Interhospital variations in the target PACs studied using hierarchical cluster analysis found that the usage and removal of PACs in H3 was distinct from those in H1 and H2. Removal efficiencies of PACs varied from highly negative or no removal (e.g., GEM and FEN), to highly positive (e.g., ZOL, NSER, QUE, CHLM, and ALP) levels, because there are various factors that can influence removal efficiencies, including input load and treatment plant configuration. Several PACs demonstrated low or negative removal rates, highlighting the importance of including metabolites in analytical methods and acknowledging their roles in pharmacological/toxicological activity. Ecological risk assessment revealed that compounds such as IBU, TRA, and CHLM pose a high risk to various aquatic species. A 100% frequency of detection of 20 target compounds in the Mid Canal water emphasized the potential concern for contamination downstream of the largest river in Sri Lanka. Furthermore, in a pandemic situation, such as the current COVID-19 issue, large quantities of pharmaceuticals are being used, but their occurrence has not been studied so far, and their environmental effects are still unknown. The pre–COVID-19 data presented in our study will be useful for comparing the ecological effects of PACs in wastewater during and after the COVID-19 pandemic. Therefore, it is indeed important to continue monitoring the presence of these compounds in wastewater, which could predict their effects on the receiving environment.

Supporting Information—The Supporting Information are available on the Wiley Online Library at https://doi.org/10.1002/etc.5212.

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Data Availability Statement—Data, associated metadata, and calculation tools are available from the corresponding author (guruge@affrc.go.jp).

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