4. Research report

4.1 Aim:

Since December 2019, the global COVID-19 pandemic has significantly impacted the utilization of pharmaceuticals and personal care products (PPCPs), leading to potential adverse effects on aquatic environments. This study aims to collect and analyze waste and surface water samples from Sri Lanka during the COVID-19 period. The research builds upon prior environmental monitoring of PPCPs conducted before the pandemic. A comparative analysis of data collected before and during COVID-19 will be undertaken to comprehend potential environmental consequences.

4.2 Objectives:

The following studies will be conducted to assess the influence of the COVID-19 pandemic on surface and wastewater in Sri Lanka:

(1) Examination of the presence of PPCPs in diverse aquatic environments.
(2) Assessment of the detrimental ecological impacts of PPCPs on the most vulnerable aquatic species.
(3) Investigation into the association between the occurrence of antimicrobials and the development of drug-resistant bacteria and resistance genes.

4.3 Materials and Methods

4.3.1 Sample collection:

In 2022, amidst the surge of the Omicron variant of COVID-19, a total of 91 samples were collected across five seasons (January, February, April, July, and December) from diverse sources. These sources included eight hospitals, four surface water sampling sites (lake, canal, and river), and a wastewater treatment plant (WWTP) in the Kandy area of Sri Lanka (Figure and Table).

4.3.2 Extraction and detection:

In this study, approximately 100 selected pharmaceuticals, including 37 antibiotics, were targeted in water samples. The analytical method employed modifications of previous procedures (Guruge et al., 2019; Tanoue et al., 2015). Briefly, samples underwent filtration using glass-fiber filters to eliminate suspended solids. A 20 mL aliquot of the filtrate was acidified with formic acid and spiked with internal standards. The sample solution went through Oasis HLB and Oasis MCX cartridges, preceded by specific preconditioning steps. After loading and washing, antimicrobial agents were eluted from the cartridges, combined, evaporated, and finally diluted for identification and quantification using LC–MS/MS. To minimize adsorption, containers and vials made of PP or PE were exclusively utilized, and acetonitrile replaced methanol in the analysis of beta-lactam antibiotics.

4.4 Results

4.4.1 Drug usage pattern in hospitals

According to epidemiological data on drug usage in the Kandy district, information gathered from the Regional Medical Supply Department (RMSD) in the Kandy district indicates a priority usage of certain drugs. The penicillin family drugs, specifically amoxicillin, cloxacillin, and Co-amoxiclav, were identified as the most frequently used. In the category of cephalosporins, cefalexin, cefuroxime, and ceftriaxone were prioritized as 1st, 2nd, and 3rd-generation options, respectively. Doxycycline emerged as the primary tetracycline drug. Among macrolides, clarithromycin, erythromycin, and azithromycin were the most commonly utilized, while Co-trimoxazole (a mixture of sulfamethoxazole and trimethoprim) was the preferred choice for sulfonamides. Ciprofloxacin stood out as the predominant fluoroquinolone. In the realm of antiparasitic and antifungal drugs, metronidazole, mebendazole, and nystatin were given priority. For psychiatric drugs, priority was accorded to carbamazepine, phenytoin, and chlorpromazine. In the cardiac drugs category, losartan, atorvastatin, and diltiazem were among the prioritized options. Non-steroidal anti-inflammatory drugs (NSAIDs) were represented by ibuprofen and diclofenac, which received high priority. At the COVID-19 Intermediate Care Center (H-8), patients were predominantly administered azithromycin, amoxicillin and ciprofloxacin antibiotics. Additionally, azithromycin was consistently used for treating all COVID-19 patients in other hospitals, suggesting its potential as a drug-marker for tracing COVID-19 related drugs in wastewater and surface waters.

4.4.2 Occurrence of antimicrobials (AMs)

Eleven drug classes of antimicrobials (AMs) comprising 37 individual drugs were chosen for this analysis. The recovery criteria were set as follows: absolute recovery exceeding 30%, an acceptable range corrected with internal standards falling between 70 – 120%, and a variation of less than 15%. For the initial analysis, two sample sets were examined. The first set, collected in January 2022, coincided with the rise of the Omicron variant of COVID-19, while the second set, collected in December 2022, was during the period when COVID-19 surveillance was not conducted in Sri Lanka. Samples were selected from the largest hospital (H-1), H-8, WWTP inlet and outlet, adjacent river, canal, and

lake.
A total of 22 compounds were identified in at least one sample; nevertheless, only 16 of these compounds have been chosen for discussion, as indicated in the table. The highest AM detected in our analysis was metronidazole (791000 ng/L), an antiparasitic drug prioritized for use, identified in the sample associated with COVID-19 patients from H-1. Additionally, ciprofloxacin and azithromycin were also present in high concentrations in the same sample collected during the surge of COVID-19 cases. A similar trend was observed in samples from H-8, where these two drugs, along with clarithromycin, exhibited concentrations several magnitudes higher than those in non-COVID samples. Notably, the concentration of azithromycin was 2620-fold higher. This data suggests that azithromycin could serve as a significant marker for tracing COVID-related drugs in environmental waters.
Our analysis also identified two beta-lactam drugs, cefuroxime and piperacillin, detected in samples from the WWTP outlet, river, and connected canal, raising the need for further investigation into why they were not present in untreated hospital wastewater.
Among other antimicrobials, doxycycline, sulfamethoxazole, and trimethoprim were found in high concentrations, aligning with their prioritization as important AMs in hospital settings.
Even though not all samples have been analyzed yet, the initial estimate of the removal efficiency of PPCPs in the WWTP appears satisfactory, ranging from 58% to 99% for sulfonamides, trimethoprim, macrolides, lincosamides, fluoroquinolones, and tetracyclines.

5. Future challenges:

Currently, remaining target PPCPs have been analyzing in rest of samples. Once all samples are analyzed, the 2nd and 3rd objectives will be carried out to emphasizes the ecological impacts of detected PPCPs together with their association for antimicrobials and occurrence of drug-resistant bacteria and resistance genes. The additional samples which were collected in December 2023 from the same locations will also be analyzed under the Lamer 2023 research program.

6. Acknowledgment:

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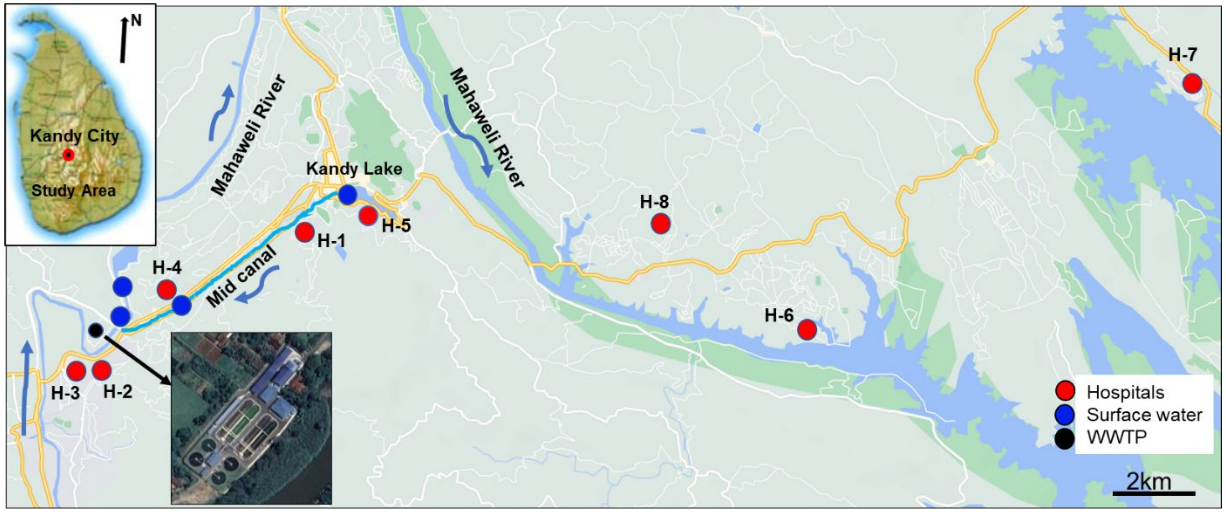

Figure. Sample locations

H-1: Largest Hospital

[samples were collected from four sites]

H-8: COVID-19 Intermediate Care Center

[samples were collected from two sites]

Table. Concentration of antimicrobials detected in aquatic samples from Kandy area-2022

	H-1-1		H-1-2		H-1-3 (covid+)	H-1-4		H-8	H-8 (covid+)		WWTP-Inlet		WWTP-Outlet		River - Before		River- After		Canal		Lake	
	January	December	January	December	January	January	December	January	January	December	January	December	January	December	January	December	January	December	January	December	January	December
Sulfapyridine	12300	119	1875	1285	<MDL	<MDL	78	<MDL	4	4735	2165	1025	825	278	6	2	3	1	49	8	<MDL	<MDL
Sulfamethoxazole	64000	525	455	235	2390	10	136	<MDL	<MDL	6550	1770	940	560	490	4	1	2	<MDL	20	5	<MDL	2
Trimethoprim	13900	310	313	221	11700	27	15	<MDL	<MDL	279	695	220	107	96	1.3	<MDL	0.5	<MDL	15	1	<MDL	<MDL
Lincomycin	174	103	23	18	16	<MDL	<MDL	<MDL	<MDL	<MDL	11	8	4	1	<MDL	<MDL	<MDL	<MDL	0.3	0.1	<MDL	0.1
Clindamycin	50500	13000	780	2310	7100	26	88	0.4	2	10	1130	530	940	361	1	1	1	0.3	13	8	1	1
Clarithromycin	11	85	685	20200	800	13	18	6	2520	<MDL	292	457	9	53	0.3	2	0.04	0.1	0.25	0.01	19	0.02
Azithromycin	126	1500	53	53	7530	4	31	4	11300	293	53	201	<MDL	4	<MDL	1	<MDL	1	<MDL	0.5	19	0.4
Vancomycin	96	32	298	1400	14	520	<MDL	<MDL	<MDL	<MDL	221	121	179	118	5	<MDL	<MDL	<MDL	63	<MDL	<MDL	<MDL
Levofloxacin/ofloxacin	295	15300	811	6020	14550	560	111	3	107	89	395	413	80	48	3	1	<MDL	<MDL	1	<MDL	2	<MDL
Ciprofloxacin	5246	2961	2906	7246	39096	3961	5246	9	4065	1980	2341	4446	120	113	8	9	<MDL	4	<MDL	<MDL	18	<MDL
Doxycyline	234	476	108	104	2350	105	22	0.4	20	11	83	170	15	29	0.4	2	<MDL	0.1	0.9	1.0	1	0.2
Fluconazole	219	149	46	67	4	398	10	1.5	1.3	6.6	138	55	173	63	4.9	2.8	2.3	1.6	25	4	3.2	3.9
Ketoconazole	16	47	<MDL	59	6	88	392	18	50	90	2	28	3	7	19	13	15	54	41	15	52	19
Metronidazole	1188	80	1933	22200	791000	13900	808	8	<MDL	<MDL	3170	299	308	548	22	18	4	6	215	270	1	1
Cefuroxime	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	12	13	24	6	9	<MDL	38	<MDL	<MDL
Piperacillin	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	8	3	286	3	4	<MDL	85	<MDL	<MDL