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The Contribution of Pharmaceutical Pollutants in Sri Lankan Waters to Antibiotic Resistance

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Introduction and Background

Assessing urban water vulnerability is vital but complex for ensuring water resilience. Integrated water management helps reduce negative social, economic, and environmental impacts while addressing the growing need for safe water (Honda et al. 2016; Kumar et al. 2019b).

The emergence of new human-made compounds, known as “emerging contaminants” is raising concerns due to their harmful effects on ecosystems and human health (Gogoi et al. 2018). Those emerging contaminants in water are a global challenge, as they are unregulated and not fully understood. Conventional wastewater treatment plants do not completely remove these pollutants. Many of the United Nations’ Sustainable Development Goals (SDGs) include reducing emerging contaminants and their discharge into water resources. Some scientists argue that these emerging pollutants may harm the SDGs and even threaten their achievement (Domingo-Echaburu et al. 2021).

Those emerging contaminants such as multi-drug resistant microbes and Pharmaceuticals and Personal Care Products (PPCPs) in urban waters have become vital in urban sustainability issues. The vulnerability related to concurrence of emerging contaminants, source identification, and indicator selection requires further study. Pharmaceuticals are chemicals used in medicines to treat or prevent illnesses, including antibiotics, painkillers, beta-blockers, and lipid regulators (**Tushara Chaminda, G.G.** et al. 2013a). Once consumed, some pharmaceuticals are transformed during metabolism while others remain unchanged before all are excreted along with urine and faeces. Potential concerns from the environmental presence of these emerging contaminants, such as pharmaceutical contaminants, include abnormal physiological processes and reproductive impairment, increased incidences of cancer, the development of antibiotic-resistant bacteria, and the potential increased toxicity of chemical mixtures (Gogoi et al. 2018). Upon discharge of treated sewage into receiving water bodies, residual pharmaceutical contaminants may be diluted or blended with contaminants from other discharge points, runoff, and seepage (**Tushara Chaminda, G.G.** et al. 2013a).

Limited data on the effects, fate, and concentrations of emerging pollutants



like pharmaceutical contaminants make regulation and management challenging for governments. While research on pharmaceutical contamination in water sources is common in developed countries, such studies remain scarce in developing nations, including Sri Lanka (Honda et al. 2011; **Tushara Chaminda, G.G.** et al. 2013b; Kumar et al. 2019b; Do et al. 2021; Kumar et al. 2021). Urban waters are becoming significant pharmaceutical contaminants' sources, but is there enough capacity to address this issue? Since the quantity, type, and patterns of pharmaceutical contaminants usage are geographically varied, their occurrence in the urban water environment may differ in Sri Lanka compared to other countries. What is the vulnerability to such emerging pollutants in the urban waters of Sri Lanka? Therefore, we have undertaken several research studies over the past six years with the following objectives.

1. Evaluate the comparative prevalence of Pharmaceuticals Contaminants and Antibiotics in Urban waters in Sri Lanka.
2. Use Pharmaceuticals Contaminants as markers to detect faecal pollution in groundwater and surface water.
3. Assess the prevalence of antibiotic-resistant bacteria (ARB), antibiotic resistance genes (ARG).

Some of our research outputs from those studies can be considered the first-ever research publications in this field regarding the Sri Lankan context. We collected water samples at the influents and effluents from sewage treatment plants (STPs), hospital discharge, river waters (Kelani and Gin), and urban canals in Galle and Colombo. Samples were analysed for pharmaceutical parameters at the laboratories in Tokyo, Japan, using LC-MS/MS coupled to a triple quadrupole mass spectrometer. Water samples were pretreated at the University of Ruhuna and preserved and frozen before transportation to Tokyo, Japan. The method quantitation limit for all analytes ranged from 0.005 to 0.15 ng/L (Do et al. 2021). We also tested for antibiotic resistance of *E. coli* bacteria in Kelani and Gin rivers, urban canals, and STPs in Galle and Colombo. The isolated *E. coli* colonies were evaluated for resistance to six antibiotics such as levofloxacin (LVX), ciprofloxacin (CIP), norfloxacin (NFX), kanamycin monosulphate (KM), tetracycline (TC), and sulfamethoxazole (ST). The Kirby-Bauer (KB) disc diffusion method, as described by (Honda et al. 2016), was employed at the Environmental Engineering Laboratory of the University of Ruhuna in Galle. Real-time quantitative reverse transcription PCR (qRT-PCR) analysis of antibiotic resistance genes (ARGs) was subsequently performed in Japan. Principal component analysis was performed to characterize the resistance patterns of the samples.

Prevalence of Pharmaceuticals in Urban Waters in Sri Lanka

We screened for 82 Pharmaceuticals from sewerage treatment plants (STPs) and detected 20 pharmaceuticals. The concentrations of detected pharmaceutical compounds in raw wastewater from the Hikkaduwa STP and hospital effluent (Karapitiya) were comparable with those frequently detected in previous studies in developed countries (Quyen et al. 2021). Acetaminophen (antipyretic) and Caffeine (stimulant) accounted for the highest total concentrations in STP influent and hospital discharge. Carbamazepine (Neuroactive drugs), Cotinine (Tobacco metabolite), Sulfamethoxazole (Antibiotic), Sulfapyridine (Antibiotic), Atenolol (Beta-blocker), and Acesulfame (Artificial sweetener) were other detected pharmaceutical parameters in raw sewerage and treated effluents of municipal STP and hospital effluent as well. Acetaminophen is a widely used over-the-counter medicine and is often detected at high concentrations in domestic wastewater (Tushara Chaminda, G.G. et al. 2013a; Tushara Chaminda, G.G. et al. 2013b). The Acetaminophen concentration of raw sewage of Hikkaduwa STP was detected to be up to $43 \mu\text{g/L}$, while Caffeine was up to $69 \mu\text{g/L}$. Despite the high concentrations, we found that Acetaminophen and Caffeine were generally removed up to 99% in the Hikkaduwa STP. Carbamazepine, Atenolol, Sulfapyridine and Sulfamethoxazole were also detected up to 0.24, 0.48, 0.47, and $0.20 \mu\text{g/L}$ in municipal STP in Hikkaduwa. Unlike acetaminophen, it was observed that Carbamazepine and other detected pharmaceutical parameters were not adequately removed during the biological treatment process in Hikkaduwa STP.

Acetaminophen is the highest concentrated pharmaceutical compound found in hospital effluent and was detected up to $124 \mu\text{g/L}$ while two antibiotics (Sulfamethoxazole and Sulfapyridine) were detected up to $2.6 \mu\text{g/L}$ indicating the current treatment process used by the Karapitiya STP was not sufficient to remove pharmaceutical compounds. The complicated composition of hospital discharge suggests a longer-term monitoring study. We also collected surface water samples from Galle municipal canals consisting of the Moragoda, Moda, and Kepu Ela, which also receive wastewater from residential areas. Our household survey found that two-thirds of the urban greywater is often discharged above municipal canals in Galle. We detected Acetaminophen and Caffeine up to $4.7 \mu\text{g/L}$ and $21.7 \mu\text{g/L}$ in urban canals within Galle city. Carbamazepine was also detected at low concentrations in those urban canals.

We collected 32 water samples (31 private and one public well) during the 2019 monsoon season from groundwater wells in the Galle area. Four pharmaceuticals at trace levels (ng/L) were detected in groundwater, suggesting that wastewater impacts the septic tanks. Caffeine was detected in 89% of the sample at a maximum concentration of 7.9 ng/L , indicating fecal pollution. Carbamazepine was detected in 42% of wells with a concentration of up to 6.9 ng/L , located close to and downstream of the septic tanks. We detected an antibiotic (Sulfamethoxa-



zole) with concentrations as low as 1.1 ng/L in groundwaters in the Galle area. We also found that Acetaminophen in groundwater was less detectable, suggesting that physical and biological processes within the septic tanks and the saturated zone limit their transfer to the shallow groundwater (Do et al. 2021). Our studies emphasise the potential impact of poor sanitation on groundwater resources and the necessity of sanitation improvement in achieving Sustainable Development Goals in developing countries like Sri Lanka (Do et al. 2021). Therefore, we suggested that a setback distance $> 18 \text{ m}$ is unlikely to protect groundwater from septic tank contamination. Thus, we recommend a more significant vertical separation from septic tanks to groundwater or installing watertight septic tanks where possible.

Pharmaceutical parameters as markers to detect pollution sources in groundwater and surface water

Our studies suggest that Acetaminophen (antipyretic), Caffeine (stimulant), Carbamazepine (Neuroactive drugs) and Acesulfame (Artificial sweetener) can be used for identifying pollutant sources in Galle City. The stimulant, Caffeine, and the artificial sweetener, Acesulfame, are included in everyday beverages, such as tea, coffee, and soft drinks, so black and grey water may contain these compounds. However, the fact that Caffeine is readily degradable and entirely metabolised in the human body suggests that black water is not a significant source and is more likely to be a grey water indicator. However, due to the persistence of Acesulfame during wastewater treatment, it can be used to generalise the impacts of domestic wastewater on surface water. Acetaminophen (antipyretic) and Carbamazepine (Neuroactive drugs) are active pharmaceutical ingredients. As expected, the Carbamazepine level was significantly lower than Acetaminophen in urban wastewater owing to its limited use. Therefore, we suggested using Acetaminophen as an indicator for identifying the impact of untreated human excretion over short distances.

Further, Carbamazepine could be a good indicator for tracking hospital residues over long distances owing to its environmental persistence (Quyen et al. 2021). Our finding of the presence of pharmaceutical contaminants in well waters indicated the vulnerability of the drinking groundwater source to faecal contaminants from the outdated sanitation system. We observed that *E. coli* and total coliform had a significant positive correlation with Caffeine concentration in groundwater. Moreover, a significant correlation was also observed between nitrate and Carbamazepine in groundwater (Do et al. 2021). These results imply that hospital wastewater treatment was not working correctly, accounting for pharmaceutical residues reaching surface water via hidden discharges connected to the drainage system.

Development of Antibiotic Resistance in Urban Waters

Our studies between October 2017 (wet season) and March 2018 (dry season) evaluated the occurrence of antibiotic-resistant bacteria (ARB) and antibiotic-resistant gene (ARG) for six antibiotics: Norfloxacin (NFX), Ciprofloxacin (CIP), Levofloxacin (LVX), Kanamycin monosulfate (KM), Tetracycline (TC), and Sulfamethoxazole (ST) in influents and effluent of sewerage treatment plant (STP) and surface waters in Colombo and Galle using KB disk diffusion method. Samples were collected from two municipal STPs from Colombo and hospital effluent from Galle. On the other hand, we collected water samples at different locations of Kelani River, Gin River, and some urban canals (Wellawatta Canals in Colombo, Moda Ela, and Moragoda Ela in Galle). For all STP samples, at least 50% of colonies showed resistance to all six antibiotics. Our antibiotic-resistant gene (ARG) screening test results were in agreement with the antibiotic resistance bacteria (ARB) test (Sovannlaksmy et al. 2020). Compared to municipal STP, the hospital effluent had more multidrug resistance due to the higher concentration of pharmaceutical products in the wastewater (Kumar et al. 2020c). As previously reported, we also noticed no significant contribution of faecal contamination was found to increase the antibiotic resistance ratio (Honda et al. 2016). *E. coli* prevalence was reduced during treatment, but the remaining bacteria could adapt to antibiotics, leading to further antibiotic resistance. Concerning some countries (India, Thailand, Poland, Ireland, Portugal, Austria, etc.), the STPs in Sri Lanka showed more antibiotic resistance and a consistent increase in antibiotic resistance after the treatment (Kumar et al. 2020c). We further noticed that antibiotic resistance was strongly correlated with fluoroquinolones in every WWTP. Fluoroquinolone-resistant genes (*aac*- (6')-*Ib-cr*, *qnrB*, *qnrS*), β - *lactams* (*ampC*), and sulphonamides (*sulI*) were detected in municipal WWTPs with additional genes for *parC* in the hospital wastewater of Sri Lanka, implying much higher resistance to fluoroquinolones, especially ciprofloxacin (Kumar et al. 2019a). On the other hand, *E. coli* bacteria isolated from Jaela STP's effluent and Galle's hospital effluents (20 m and 50 m downstream) exhibited 100% antibiotic resistance.

We further observed that almost all sampling points in Kelani and Gin rivers and canals contained *E. coli* strains that showed resistance to more than four antibiotic multidrug resistance, alarming health concerns in the future. Our studies suggested high antibiotic resistance in the Kelani River (up to 90%) compared to the Gin River (up to 40%). Wellawatta Canals, Moda Ela, and Moragoda Ela showed more antibiotic-resistant *E. coli* than Gin and Kelani Rivers owing to more pollution and lower flow rates in canals (Kumar et al. 2020b). We found that the resistance percentage for older antibiotics like Sulfamethoxazole (ST) > Tetracycline (TC) was higher than the newer antibiotics in the Kelani River. Further, for non-fluoroquinolone (KM, ST, and TC), higher resistance was observed at down-



stream locations in Kelani River compared to upstream locations, which probably indicates the antibiotic use pattern affecting the resistance. On the other hand, the resistance percentage of fluoroquinolone (LVX, CIP, and NFX) decreased from upstream to downstream in the Kelani River (Kumar et al. 2020a).

The investigation into ARGs in Sri Lankan urban rivers in Southern Province, revealed two distinct distribution patterns, providing insights into their likely sources and dissemination. The *mphA* gene, which provides macrolide resistance, was found at higher concentrations in heavily polluted urban waters like Galle's Moragoda Canal. The *blaIMP* carbapenemase gene, a marker of hospital waste and clinical concern, was found in all three waters in Galle (Moragoda Ela, Gin River, and Kepu Ela) (Shayan 2025).

Conclusions and Recommendations

Our results imply that the urban drainage systems in Sri Lanka receive both domestic graywater and human excretion, likely due to insufficient on-site sanitation systems. Further, we noticed the potential impact of poor sanitation on groundwater resources and the necessity of sanitation improvement in achieving Sustainable Development Goals in developing countries like Sri Lanka. We detected around 20 pharmaceuticals in urban waters in Sri Lanka. Acetaminophen was dominant in hospital discharge (up to $124 \mu g/L$), while caffeine was the most significant contributor to STP influents (up to $69 \mu g/L$). These results imply that hospital wastewater treatment was not working correctly, accounting for pharmaceutical residues reaching surface water via hidden discharges connected to the drainage system. We suggest using caffeine and acetaminophen as indicators for identifying the impact of untreated human excretion over short distances. Further, Carbamazepine could be a good indicator for tracking hospital residues over long distances owing to its environmental persistence. The investigation into ARGs in Sri Lankan urban rivers in Southern Province, revealed two distinct distribution patterns, providing insights into their likely sources and dissemination. Further, we noticed that 50 to 100% of antibiotic resistance in urban waters of Sri Lanka was comparatively higher than that of other countries, alarming that multidrug-resistant had become a significant concern for community health. Our studies imply that urbanization, land use, and lifestyle are the three most critical factors governing multidrug resistance. We strongly recommend continuous monitoring of the urban water sources for pollution load, as ongoing assessments are required for sustainable water management.

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urban waters, antibiotic-resistant bacteria and genes in urban waters, virus indicators in water and soil, sustainable water use in urban areas, and green building technology for water optimization and carbon footprint reduction. Prof. Tushara Chaminda has established an impressive publication record, authoring over 200 scientific works, including international journal articles, book chapters, and presentations at both international and local conferences. By 2025, his research had accumulated over 2,300 citations and achieved an H-index of 22. His research excellence is further recognized by numerous international and local awards, including the prestigious international “Award for Asian Young Professional in Water Research” (2012), the Sri Lanka President’s Awards for Scientific Publication (awarded on 4 occasions), Merit awards for Scientific Publications by the National Research Council (5 awards), the Vice-Chancellor’s Award for Most Outstanding Scholar at the University of Ruhuna (2023), and the Tier 4 star—Highest Research status award by the University of Ruhuna in 2024. Furthermore, he serves as Editor-in-Chief and Editor for several international and local academic journals.

