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Contamination of Surface Water and River Sediments by Antibiotic and Antiretroviral Drug Cocktails in Low and Middle-Income Countries: Occurrence, Risk and Mitigation Strategies

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Abstract: Presence of antimicrobial cocktails in the hydrological cycles is of interest because of their potential to mediate antimicrobial resistance within the natural environment. In this study, we determined the concentrations of selected antibiotics and antiretroviral drugs (ARVDs) in wastewater treatment plant (WWTP) effluent, effluent suspended particulate matter (SPM), surface waters and river sediments in Kenya in order to determine the extent of pollution within the sampled environment. Target analysis for the most common antibiotics and ARVDs was done. Sulfamethoxazole (SMX), ciprofloxacin (CIP), trimethoprim (TMP), norfloxacin (NOR), zidovidine (ZDV), lamivudine (3TC) and nevirapine (NVP) were analyzed using LC-ESI-MS/MS. Effluent aqueous phase had concentrations ranging between 1.2 μg L^{-1} to 956.4 μg L^{-1} while the effluent SPM showed higher concentrations, ranging between 2.19 mg Kg^{-1} and 82.26 mg Kg^{-1}. This study shows emission of active pharmaceutical ingredients (APIs) from WWTP to the environment mainly occurs via the SPM phase, which is usually overlooked in environmental analyses. Concentrations in surface waters and river sediments ranged between 1.1 μg L^{-1} to 228 μg L^{-1} and 11 μg Kg^{-1} to 4125 μg Kg^{-1} respectively. ARVDs occurred at consistently higher concentrations than antibiotics in both the aqueous and solid samples. The wastewater treatment plants and lagoons where sludge degradation should occur, are sources of active pharmaceutical ingredients (APIs) including transformational products, nutrients and organic matter that are released back to the aqueous phase.

Keywords: wastewater; antibiotics; antiretroviral drugs; antimicrobial resistance; suspended particulate matter; sediments

1. Introduction

Pollution by pharmaceutical micropollutants is an emerging area of concern. The effect of cocktails of active pharmaceutical ingredients (APIs) to non-target organisms is largely unknown [1]. Cocktails of APIs and their active metabolites enter the environment due to incomplete removal by wastewater treatment plants (WWTPs) after human and veterinary use. Indeed, centralized wastewater treatment plants are point sources of emerging micropollutants, especially active pharmaceutical ingredients (APIs) into the environment [2]. This happens because pharmaceuticals are not completely metabolized in the body and are excreted in urine and fecal matter, as either parent

compounds or as pharmacologically active metabolites [3–6]. In water-based sanitation, the active ingredients and their metabolites undergo dilution with large volumes of water as they are flushed down the drain, where they mix with other household chemicals and personal care products. Household, hospital and industrial wastewater as well as a runoff water mix and are channeled into the centralized WWTPs. Dilution of the organic micropollutants to very low concentrations (ng L^{-1} or below) occurs, which cannot be effectively removed from the WWTP, making them ubiquitously present in the water bodies [7]. Pseudo-persistent APIs in the environment have the potential to mediate antimicrobial resistance among the environmental pathogenic microorganism [8]. Effluent from healthcare facilities, WWTPs, pharmaceutical and other industries—especially in low- and middle-income countries—is insufficiently regulated [9]. WWTPs are beneficial for public health; however, they act as sinks to important nutrients such as phosphorus and nitrogen as well as minerals. In wastewater, plants where the activated sludge removal, treatment and discharge into landmines or fields occurs, the flow of adsorbed recalcitrant micropollutants happens between aqueous phase to terrestrial systems. Wastewater treatment plants such as lagoons, anaerobic and aerobic ponds and trickling filters in which the excess sludge is meant to decompose, are where the nutrients and recalcitrant matter including micropollutants are desorbed and released from the sludge into the effluent and eventually into the receiving water bodies, where they potentially cause eutrophication and stress to aquatic organisms [10,11].

Previous research work on this topic has focused on the aqueous phase, but here we also report data on occurrence of active pharmaceutical ingredient (API) cocktails in suspended particulate matter (SPM) and river sediments. This study was aimed at assessing the levels of selected common antibiotics; sulfametoxazole (SMX), ciprofloxacin (CIP), trimethoprim (TMP), norfloxacin (NOR) and antiretroviral drugs; zidovidine (ZDV), lamivudine (3TC) and nevirapine (NVP) in the effluent, SPM surface water and river sediments of selected sampling sites in Kenya.

2. Materials and Methods

2.1. Study Area and Sample Collection

Effluent surface water grab and river sediment upstream and downstream of the effluent discharge point from the wastewater treatment plant (WWTP) was collected in Machakos town, Kenya. The WWTP Machakos in Machakos employs waste stabilization ponds for wastewater treatment. Machakos town is the administrative town of the larger Machakos County. The Machakos town constituency has a population of 50,753, with a WWTP serving 7.6% of the population while 13.1% and 55.2% of the population use septic tanks and pit latrines, respectively [12].

Two sampling campaigns in January and September 2019 were carried out. September is usually a very dry month and most arid and semi-arid areas suffer drought, affecting the flow rates into the treatment plant as well as in the rivers. The river Mitheu, which receives the effluent from the WWTP, was almost drying up and the flowing waters were contaminated with raw sewage judging by the odor and appearance. Generally, the water volumes in the rivers were significantly decreased during the September sampling as compared to the January sampling. The physicochemical characteristics of the samples are shown in Table 1. The effluent composite sample was constituted by combining the eight hourly grab samples into a large container from which duplicate 1-L representative samples were drawn and taken to the laboratory for further processing. Duplicate grab 1-L river water samples were collected at approximately 500 m upstream and downstream of the effluent discharge point. River sediment samples were collected at similar points corresponding to the aqueous samples and dried indoors at room temperature (25 °C). The sample collection protocols are described in detail in our previous publication [13]. The suspended particulate matter was obtained by successive filtration of 100 mL of the aqueous sample through a Whatman GF/D (2.7 μm) and GF/F (0.7 μm) filter papers. The filter papers were dried at room temperature (25 °C) and processed similarly to the sediment samples.

Table 1. Psychochemical characteristics of the effluent and surface water samples showing the pH, temperature, electrical conductivity, total dissolved solids and total suspended solids. ES = electrical conductivity, TDS = total dissolved solids and TSS = total suspended solids.

Sample	pH	Temp (°C)	EC (dS/m)	TDS (ppm)	TSS (mg L^{-1})
Effluent	7.88	30.2	5610	3.73	72.8
Surface water	6.36	27.6	1140	2.86	66.4

2.2. Chemicals and Standards

All pharmaceutical standards and corresponding isotope-labelled internal standards were of >99% purity. All the isotopically labeled internal standards were purchased from Alsachim (Illkirch Graffenstaden, France) apart from ($^{2}H_{9}$)-TMP which was purchased from Sigma-Aldrich (Steinheim, Germany). HPLC grade acetonitrile and methanol were purchased from Merck (Darmstadt, Germany), ammonium hydroxide (25%) solution was purchased from Merck (Overijse, Belgium), formic acid and formic acid (98%) from Fluka (Munich, Germany). Stock solutions and the working standards were prepared and stored at +4 °C in amber vials.

2.3. Sample Cleanup and Pre-Concentration

Environmental sample cleanup and pre-concentration for aqueous samples was carried out following the protocol described by Ngumba et al., (2016) [14] without modifications. The river sediment samples were analyzed by the method described elsewhere with some modifications [15].

Briefly, 1 g of dried sediment was weighed into a 50 mL centrifuge tube (VWR), spiked with 40 µL of 10 mg L^{-1} mixture of isotopically labeled internal standards, and allowed to equilibrate for ~30 min at room temperature. Extracting solvent (methanol:water, 80:20) was added (6 mL) to the mixture and vortexed for one minute. The mixture was sonicated for 20 min using a ultrasonic bath sonicator, VWR USC 1200TH, Leicestershire, UK. Extracts were centrifuged at 4500 rpm with SANYO HARRIER18/80, London, UK for 10 min and the supernatant collected in a 15 mL glass Kimax® test tubes. A repeat extraction using 6 mL of 100% methanol was done and extracts were combined into the 15 mL tube. Evaporation under a stream of nitrogen to approximately 1 mL followed and reconstituted to 10 mL using milli-Q water. The reconstituted sample cleanup followed the protocol described by Ngumba et al., 2016 [16] for surface and wastewater samples.

2.4. Instrumental Analysis

An isotope dilution method was employed in the analysis of all the target compounds. Eight-point calibration curves were prepared for each analyte by plotting response ratio of the peak area of analyte divided by peak area of internal standard (*y*-axis) against concentration ratio of the analyte divided by concentration of internal standard (*x*-axis). The multiple reaction monitoring parameters are shown in Table 2.

Table 2. The multiple reaction monitoring parameters. SMX = sulfamethoxazole, CIP = ciprofloxacin, TMP = trimethoprim, NOR = norfloxacin, ZDV = zidovidine, 3TC = lamivudine, NVP = nevirapine.

Target Compound	RT[a]	Precursor Ion $[M + H]^+$ (m/z) (CV)[b]	Quantifier Ion (m/z) (CE)[c]	Qualifier Ion (CE)
3TC	1.5	229.9 (17)	112.0 (18)	95.0 (29)
ZDV	2.3	268.2 (16)	127.0 (17)	110.1 (25)
NVP	4.1	267.2 (40)	226.2 (29)	198 (29)
CIP	2.2	332.1 (34)	288.0 (19)	314.1 (19)
TMP	2.2	291.1 (34)	123.0 (19)	230.0 (19)
NOR	2.1	320.3 (30)	276.0 (18)	302.0 (25)
SMX	5.1	254.0 (28)	156.0 (18)	108.0 (17)

[a] RT retention time. [b] CV collision voltage [c] CE collision energy.

APIs were analyzed using a Quattro micro tandem mass spectrometer interfaced with a waters alliance 2975 liquid chromatograph (LC, Milford, MA, USA). The C_{18} reversed-phase column used was (3.5 μm × 2.1 mm × 100 mm Xbridge™) fitted with a 2.1 mm × 5 mm Vanguard® and pre-column was used for separation. Gradient elution method with Formic acid (0.1%) in water and acetonitrile (100%) was used as the mobile phase. Multiple reaction monitoring (MRM) in positive ion mode was used for the determination of the analytes. The multiresidue method for trace level analysis of antibiotics and antiretroviral drugs previously published in our research group by Ngumba, Kosunen et al. (2016) [16] was used without modification.

3. Results

3.1. Instrumental Analysis Results

Table 3 shows the LC-MS/MS-ESI method qualification results. All the target compounds were detected in all the samples with the limit of detection ranging between 3 ng L^{-1} and 18 ng L^{-1}.

Table 3. LC-MS/MS-ESI method qualification results. API = active pharmaceutical ingredient, ILIS = isotopically labelled internal standard, DF = detection frequency, LOQ = limit of quantification, SMX = sulfamethoxazole, CIP = ciprofloxacin, TMP = trimethoprim, NOR = norfloxacin, ZDV = zidovidine, 3TC = lamivudine, NVP = nevirapine.

API	ILIS	r^2	% Recovery (RSD)	DF (%)	LOQ ng L^{-1}
NOR	($^2H_8$)-CIP	0.996	92.6 (3.2)	100	12
TMP	($^2H_9$)-TMP	0.999	111.3 (4.1)	100	9
CIP	($^2H_8$)-CIP	0.993	84.3 (8.3)	100	10
SMX	($^2H_4$)-SMX	0.997	101 (7.2)	100	17
3TC	($^{13}C^2H_2$ $^{15}N_2$)-3TC	0.993	98.8 (3.7)	100	15
ZDV	($^{13}C^2H_3$)-ZDV	0.988	98.7 (19.4)	100	53
NVP	($^2H_4$)-NVP	0.989	87.7 (9.3)	100	19

3.2. Occurrence of API Cocktails in the Effluent, SPM, Surface Water and River Sediments

Prevalence of antibiotics and antiretroviral drug cocktails in the effluent, effluent SPM, surface waters and sediments are shown Table 4, respectively. In the antibiotic category, SMX was predominant in the aqueous phase with a concentration range of 96 μg L^{-1} and 142 μg L^{-1} measured approximately 500 m upstream and downstream to the effluent discharge point. ARVDs were also ubiquitously present in the aqueous samples with 3TC occurring twice as much as SMX in the surface waters. The concentration of APIs in the effluent discharged into the river ranged between 1.4 μg L^{-1} and 956.4 μg L^{-1} with 3TC and SMX having the highest concentration. APIs in the effluent SPM and the river sediments occurred in μg kg^{-1} to mg kg^{-1} levels as shown in Table 4. These results indicate the effluent SPM is the major pathway for emission of APIs from the WWTP into the receiving water.

Table 4. Prevalence of antibiotics and antiretroviral cocktails in effluent, SPM, surface water and river sediments in the September sampling. SPM = suspended particulate matter, SMX = Sulfamethoxazole, CIP = ciprofloxacin, TMP = trimethoprim, NOR = norfloxacin, ZDV = zidovidine, 3TC = lamivudine, NVP = nevirapine. (sd, n = 3) PNEC = compound specific predicted no effect concentration for antimicrobial resistance selection, n.a = Not available.

Compound	Effluent Aqueous Phase	Effluent SPM Phase	Water µg L^{-1}		Sediments µg kg^{-1}		PNEC [15]
	µg L^{-1}	µg kg^{-1}	Upstream 500 M	Downstream 500 M	Upstream 500 M	Downstream 500 M	µg L^{-1}
NOR	4.2 (0.8)	82,267 (559)	1.6 (0.4)	4.9 (1.2)	776 (22)	248 (35)	0.5
TMP	15.8 (1.1)	3080 (845)	3.8 (1.2)	4.4 (1.5)	11 (3.2)	90 (19)	0.5
CIP	5.3 (0.6)	5017 (344)	2.5 (0.9)	2.8 (1.1)	4125 (236)	1275 (67)	0.064
SMX	956.4 (9.4)	23,448 (1959)	96.9 (4.6)	142.6 (8.3)	542 (13)	896 (25)	16
3TC	847.1 (25.3)	69,681 (5824)	219.6 (16.9)	228.3 (11)	491 (18.2)	107 (12)	n.a
ZDV	1.4 (1)	3336 (119)	2.1 (1.3)	1.1 (0.9)	510 (40)	118 (18)	n.a
NVP	9.5 (2.2)	3214 (146)	0.9 (0.4)	2.3 (1)	95 (14)	101 (11)	n.a

Data from the two sampling campaigns showed significant variability, with SMX and 3TC dominating. This could mainly be attributed to the drought situation during the September sampling, whereby the receiving river was almost drying up. The seasonal variation of the January and September sampling campaign results are illustrated in Figure 1.

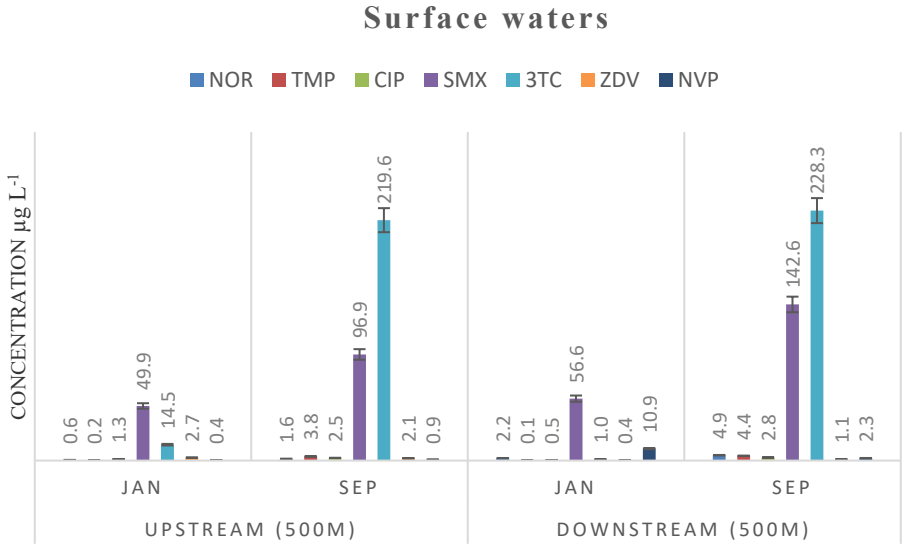

Figure 1. Seasonal variation of APIs in surface waters.

4. Discussion

4.1. Cocktails of APIs in the Natural Environment within Low- and Medium-Income Countries

This study confirms the presence of antibiotic and ARVD cocktails in the environmental samples. Concentrations of sulfamethoxazole (SMX), ciprofloxacin (CIP), trimethoprim (TMP), norfloxacin (NOR), zidovidine (ZDV), lamivudine (3TC) and nevirapine (NVP) in surface water and sediments were analyzed using a robust LC-ESI-MS/MS method. Ubiquitous detection of the all target APIs in all the collected representative samples was reported. This indicates the emission of substantial amounts of the residual antibiotics and ARVDs into the environment within the sampling areas and especially through the SPM. The measured concentrations of APIs upstream of the WWTP discharge point signifies non-point loading. Furthermore, effluent from WWTPs are considered point sources of APIs because they cannot completely remove pharmaceuticals and other personal care products within the treatment process [17,18]. Sorption of API to the SPM within the WWTP effluent

is a pathway of emission of hydrophobic micropollutants to surface waters and river sediments. Active ingredients adsorbed into the SPM enriches the sediments. Resuspension of adsorbed compounds into the aqueous phase due to biotic and abiotic activity can maintain pseudo persistency of organic micropollutants [19,20].

This can particularly occur in WWTPs where removal of sludge does not exist. The accumulated and formed sludge decomposes and the nutrients and recalcitrant matter ends up as effluent and thus spills into the receiving water bodies [21]. Other point sources include directly discharged human waste into the water bodies, because ingested drugs do not metabolize fully in the body and excretion occurs in urine and fecal matter as a parent compound or active metabolites [22,23].

The relatively higher concentration levels of APIs measured upstream of the effluent discharge point compared to the downstream samples could be attributed to the direct discharge of untreated wastewater into water bodies from informal settlements, illegal health clinics or from veterinary use. Furthermore, effluent from the WWTP is a major emission source of API into the receiving waters, with the SPM phase accounting for the bulk of the APIs emitted into the receiving waters as compared to the aqueous phase. Reduced flow rates of the surface waters due to drought in the sampling area in September indicates a lack of sufficient dilution of the WWTP effluent, thereby recording relatively high concentrations downstream of the discharge point. In most of the Kenyan towns, the centralized sewerage system covers 7.6% of the population, with the rest of the population using other sanitation solutions such as pit latrines [12]. Rapidly developing informal settlements within urban towns without a proper sanitation system increases the probability of discharge of raw sewage to surface waters.

HIV and AIDS remain a major public health issue of concern with an estimated 770,000 global fatalities in 2018. Out of the approximate 37.9 million people living with HIV/AIDS worldwide, 25.7 million are in the African region, out of which 16.3 are on lifelong antiretroviral therapy (ART). As of 2018, Kenya had approximately 1.49 million patients of which 75% were on ART, while in the same period South Africa had 7.7 million patients with approximately 62% of adults on ART. [24,25]. In 2015, it was estimated that 159,000 Kg of ARVDs reach water bodies annually in South Africa [26]. Prevalence of ARVDs in South African surface waters, ranging between 0.407 μg L^{-1} to 0.973 μg L^{-1} [27], 0.003 μg L^{-1} to 0.0067 μg L^{-1} [26] and 0.0046 μg L^{-1} to 34 μg L^{-1} [28] was reported.

Recent studies done in Kenyan surface waters have reported concentration values ranging from 6 μg L^{-1} to 167 μg L^{-1} [29], 0.5 to 1 μg L^{-1} [30] and 0.5 μg L^{-1} to 7.6 μg L^{-1} [14] for ZDV, NVP and 3TC. These results were in the same order of magnitude as the results reported in this study. Although environmental data on residual API is still scanty in developing countries, results reported by other recent studies done elsewhere on the African continent are shown in Table 5.

Table 5. Occurrence of antibiotic and antiretroviral drug residues in selected African surface waters and WWTP effluents. <LOQ = below limit of quantification, <MQL = below method quantification limit, n.d. = not detected.

Category	Compound	Sample	Concentration Range μg L^{-1}	Country	Ref.
Antibiotics	Sulfamethoxazole	surface waters	<LOQ to 9.64	Ghana	[31]
		surface waters	<LOQ to 49.56	Kenya	
		surface waters	0.511 to 53.83	Mozambique	
		surface waters	0.0033 to 10.57	South Africa	
		surface waters	11.25	Kenya	[32]
		effluent/surface water	<MQL to 0.019	Egypt	[33]
		surface water	<0.01 to 1.5	Nigeria	[34]
	Trimethoprim	surface waters	0.014 to 1.37	Ghana	[31]
		surface waters	<LOQ to 11.38	Kenya	
		surface waters	0.31 to 6.22	Mozambique	

		surface waters	0.004 to 5.88	South Africa	
		surface water	3.35	Kenya	[32]
		surface water	<0.01 to 0.4	Nigeria	[34]
		effluent/surface water	0.21 to 1.06	Egypt	[33]
	Ciprofloxacin	surface water	0.51 to 14.33	South Africa, Ghana, Kenya	[17]
ARVDs	Zidovudine	effluent/surface water	n.d. to 5.3	South Africa	[35]
		effluent	12.1 to 20.13	Kenya	[36]
	Nevirapine	effluent/surface water	<LOQ to 0.28	South Africa	[35]
		effluent	0.0053 to 3.3	Kenya	[36]
	Lamivudine	effluent/surface water	0.13 to 20.93	South Africa	[35]
		effluent	0.0325 to 60.68	Kenya	[36]

4.2. Risk of APIs in the Environment

Measured environmental concentrations of APIs shown are above the compound-specific no-effect concentrations and thus can affect non-target environmental microorganisms and aquatic life [13]. This could result in mediation of resistance selection in pathogenic microorganisms within the natural environment, resulting in antibiotic resistant bacteria (ARB) and antibiotic resistant genes (ARG). WWTPs were identified as point sources of ARBs and ARGs [37]. Antimicrobial resistance is a threat to global public health and can affect anybody in any part of the world. Resistant pathogens developed in the natural environment are harder to treat using available antimicrobials, and hence their infections can lead to an increased cost of treatment, lengthy hospitalization periods and eventually death. Pharmaceutical mixtures within the environment can have additive effects even though the risk of individual compounds could be negligible. For instance, antibiotic drug combinations designed to work synergistically, such as TMP-SMX (co-trimoxazole) with a combination ratio of 1:5 [38]. These combination ratios can also exist within natural environments, where their synergistic activity continues to act in the environmental microorganism, a precursor for antimicrobial resistance selection. Measured environmental concentrations in this study were consistently higher in the river sediment phase as compared to the surface water. This could mean that the risk of resistance selection could be greatest in the sediment phase [14]. These phases were commonly overlooked in previous studies.

Similarly to bacteria, viruses can evolve resistance against antiviral drugs, especially in instances where there is a co-existence of the virus to be treated with the antiviral drug [36]. More studies on the development of antiviral resistance is needed. Resistant infections kill approximately 58,000 newborn children in India every year [39]. Over 2.8 million resistant infections occur yearly in the United States of America, resulting in over 35,000 deaths each year [40]. In Kenya, approximately 50,000 people die each year due to multidrug-resistant tuberculosis [41]. At a time when antimicrobial resistance causes major problems in healthcare and new viral diseases emerge, it is central to understand antimicrobial contamination in the environment.

5. Conclusions

This study determined the prevalence and concentration of antibiotic and antiretroviral drug cocktails in the effluent, SPM, surface waters and river sediments of selected sampling areas in Kenya. To the best of our knowledge, this is the first study to report the occurrence of APIs in the SPM phase

within Kenyan WWTP effluents. The results indicate that SPM is an important phase for consideration in the determination of emission of micropollutants from WWTPs. Surface waters and sediments were found to be contaminated with elevated levels of the target compounds. APIs in the environment can have effects on public health on a global scale. Decentralized sanitation solutions, especially in informal settlements in the peri-urban areas, can help mitigate the direct discharge of raw sewage into surface waters. Sustainable sanitation solutions aimed at separating the urine at source are recommended, since urine is a point source of human pharmaceuticals.

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