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**Occurrence of antibiotics and risk of antibiotic resistance evolution in selected Kenyan
wastewaters, surface waters and sediments**

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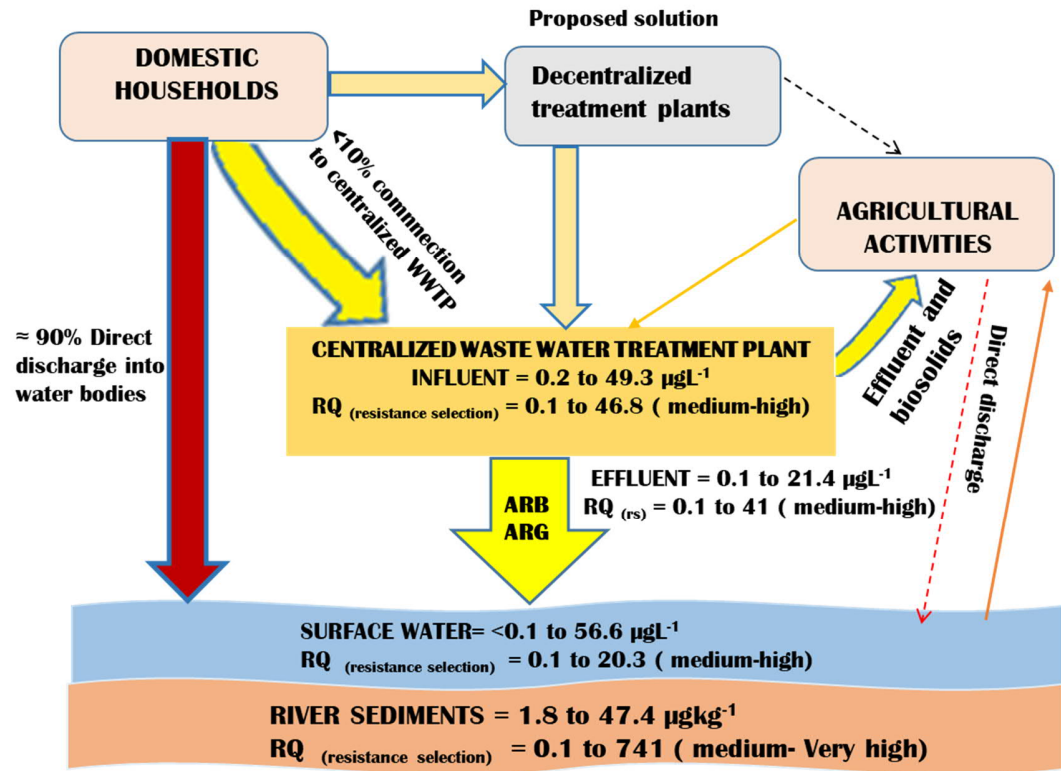
ABSTRACT

Active pharmaceutical ingredients, especially antibiotics, are micropollutants whose continuous flow into hydrological cycles has the potential to mediate antibiotic resistance in the environment and cause toxicity to sensitive organisms. Here, we investigated the levels of selected antibiotics in four wastewater treatment plants and the receiving water bodies. The measured environmental concentrations were compared with the proposed compound-specific predicted no-effect concentration for resistance selection values. The concentration of doxycycline, amoxicillin, sulfamethoxazole, trimethoprim, ciprofloxacin and norfloxacin within the influents, effluents, surface waters and river sediments ranged between 0.2 and 49.3 μgL^{-1} , 0.1 to 21.4 μgL^{-1} ; < 0.1 and 56.6 μgL^{-1} ; and 1.8 and 47.4 μgkg^{-1} , respectively. Compared to the effluent concentrations, the surface waters upstream and downstream one of the four studied treatment plants showed two to five times higher concentrations of ciprofloxacin, norfloxacin and sulfamethoxazole. The risk quotient for bacterial resistance selection in effluent and surface water ranged between <0.1 and 53, indicating a medium to high risk of antibiotic resistance developing within the study areas. Therefore, risk mitigation and prevention strategies are a matter of priority in the affected areas.

Keywords

Antibiotics; wastewater; antimicrobial resistance; antibiotic resistance evolution, risk assessment.

Graphical Abstract



Highlights

- Direct discharge of wastewater contribute to flux of antibiotics in rivers
- zero or negative pharmaceutical removal efficiencies measured in WWTP's
- Residual antibiotics occurred above predicted no-effect concentrations
- Decentralized sanitation solutions are proposed for risk control
- Risk of evolution of antibiotic resistance greatest in wastewater and river sediments

1. Introduction

Consumption of antibiotics has increased globally (Klein et al., 2018). This is due to increased disease burden, increased availability, especially of over-the-counter prescriptions, and increased resistance of pathogenic bacteria to the available antimicrobial agents (Gelband et al., 2015; Klein et al., 2018; Van Boeckel et al., 2014). The occurrence, fate and removal of active pharmaceutical ingredients (APIs), especially antibiotics, in hydrological cycles is an environmental pollution issue of global concern (aus der Beek et al., 2016; Daughton, 2016). The presence of pharmaceuticals in aquatic environments is especially high in developing countries. Studies across Africa have reported varying concentrations of common antibiotics ranging from ngL^{-1} to several orders of magnitude higher. According to a global review of API prevalence in the hydrological cycles, Europe and North America indicated relatively low prevalence (aus der Beek et al., 2016) compared with many developing countries, especially in Africa (Madikizela et al., 2017). In Kenya, APIs has been assessed in only a few studies, covering the Nairobi river basin, Nzoia river basin and Kisumu (K'oreje et al., 2018, 2016; Ngumba et al., 2016). However, the prevalence of environmental residual antibiotics in most parts of the country remains unknown. High population densities in urban and peri-urban areas, characterized by informal settlements, lack of proper sanitation facilities and high prevalence of disease (especially tuberculosis and HIV/AIDS) indicate the need to systematically assess the presence of pharmaceuticals in the environment. The majority of pharmaceuticals do not metabolize completely and therefore are excreted into the environment either in their original form or as pharmacologically active metabolites or transformational products (Carvalho and Santos., 2016a). Depending on the category of the compound, 50–90% of ingested APIs are excreted through urine (Kümmerer, 2009; Tran et al., 2016). These APIs and their active metabolites flow into the hydrological cycles by direct

discharge into the environment or through wastewater treatment plants (Kümmerer, 2008; Luo et al., 2014; Matongo et al., 2015; Zhang et al., 2015).

The presence of antibiotics in the environment at levels below therapeutic concentration may catalyze the ability of bacteria to develop antibiotic resistance (Kümmerer, 2003). As such, an environmental concentration of antimicrobials at subinhibitory levels favors the growth of both resistant and susceptible bacterial genotypes (Khan et al., 2017). These lower concentrations give competitive advantage to the growth of resistant strains (Andersson and Hughes, 2014). This may lead to the selection of highly resistant bacteria which present a greater management challenge (Li et al., 2016). Antimicrobial resistance (AMR) in wastewater, surface and treated water has been reported in various studies (Prestinaci et al., 2015; Sabri et al., 2018; Sobsey et al., 2014). The World Health Organization (WHO) has previously pronounced AMR a threat to global health (WHO, 2016).

This study was undertaken to determine the prevalence of selected antibiotic residues in the wastewater, surface water and river sediments from three counties in Kenya. Environmental sample processing and trace level analysis was carried out using a liquid chromatography electrospray ionization tandem mass spectrometer (LC-ESI-MS/MS) according to methods published by Ngumba et al. (2016) for liquid samples and Al-Khazrajy and Alistair (2017) for river sediments. Furthermore, we carried out risk assessments for resistance selection, based on the compound-specific predicted no-effect concentrations (PNEC) for resistance selection values proposed by Bengtsson-Palme and Larsson (2016). The PNEC for resistance selection was calculated based on the European Committee for Antimicrobial Susceptibility Testing (EUCAST) database for multiple genera and families of pathogenic microorganisms.

2. Materials and methods

2.1 Study area and sample collection

A five-day sampling campaign was carried out in the administrative towns of the counties of Machakos, Nyeri and Meru in the Republic of Kenya. A total of four wastewater treatment plants were sampled altogether: three wastewater stabilization ponds Machakos (WWTP1), Gateei in Nyeri (WWTP 2), Meru (WWTP 4), and one trickling filter treatment plant in Kangemi (WWTP 3) Nyeri County. Machakos County is situated 80 km southeast of Nairobi while Nyeri and Meru counties are located in the Mount Kenya region, approximately 150 km and 250 km north of the capital city, Nairobi. The selected sampling area demographics are shown in Table 1. Currently, the actual number of inhabitants served by these treatment plants cannot be accurately estimated due to the various informal settlements mushrooming within the vicinity of the sewer line and the illegal connections to it. Furthermore, wastewater soak pits, septic tanks and pit latrines are frequently found in these areas. Sampling coordinates for all the sampling spots are provided in Table S1 in the supplementary information.

Table 1: The population, percentage access to improved sources of water, sanitation and sewerage system for the selected sampling areas according to the Kenya National Bureau of Statistics (2013).

Location	Population	Access to improved sources of water (%)	Access to improved sanitation (%)	Access to sewerage system (%)
Machakos town	195,029	42.2	61	5.7
Nyeri town	111,656	85	66	16.5
Meru town	144,275	79.5	92.2	3.4

Sampling was done in January 2019, which is usually a dry month preceding short rains. Four different waste water treatment plants (WWTPs) and the rivers to which they discharge were sampled, as shown in Figure 1. Hourly 1L grab samples were collected from the WWTPs influent and effluent over a period of 8 h with 60 min interval and samples pooled to get representative 1 L composite samples. Duplicate 1L river water samples were collected approximately 200 m and 2 km upstream and downstream of the effluent discharge points. Sediment samples were also collected at a depth of approximately 5 cm from all water sampling points and air dried indoors at room temperature (25 °C).

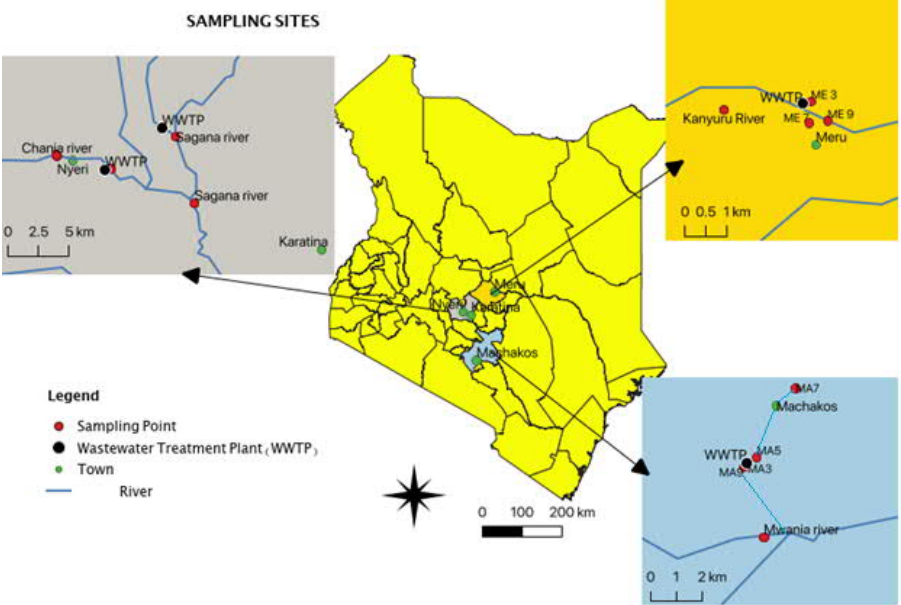


Figure 1: The map of Kenya and the extrapolated sampling sites in Machakos, Meru and Nyeri

2.2 Chemicals and standards

The pharmaceutical standards used were of >99% purity and obtained from Sigma Aldrich (US). The physicochemical properties of the standards, including their structure and CAS registry numbers, are indicated in Table S2 of the supplementary information. All the isotopically labeled internal standards were purchased from Alsachim (France) apart from [²H₉]-TMP which was

purchased from Sigma-Aldrich (Steinheim, Germany). HPLC grade acetonitrile and methanol were purchased from Merck (Germany), ammonium hydroxide (25%) solution was purchased from Merck (Belgium), formic acid and formic acid (98%) from Fluka (Germany). Stock solutions were prepared as outlined by Ngumba et al. (2016) and stored at +4 °C in amber vials.

2.3 Sample extraction

200 mL duplicate sub-samples were measured from the pooled sample and 40 µL of 10 mgL⁻¹ isotopically labelled mixed standard was added to each before processing. Samples were filtered through a 47mm GF/F (0.7 µm) glass filter followed by solid-phase extraction using Oasis HLB 6 cc (200 mg) cartridges. The extraction and analytical method developed by Ngumba et al. (2016) was used for the liquid samples. The sample concentration of the target compounds doxycycline (DOX), amoxicillin (AMO), sulfamethoxazole (SMX), trimethoprim (TMP), ciprofloxacin (CIP) and norfloxacin (NOR) was measured. Target compounds were extracted from the sediment samples using an ultrasonic bath. As outlined by Al-Khazrajy and Alistair (2017), 5 g of the air-dried sediment samples was extracted and the extracts subjected to the solid-phase extraction process. In brief, the HLB cartridges were conditioned with 6 mL of methanol followed by 6 mL of Milli-Q ultrapure water at a flow rate of 5 mL/min⁻¹. Samples spiked with isotopically labelled internal standards were loaded at the same flow rate, after which the target compounds of interest were eluted with 4 mL of 50:50 acetonitrile-methanol solution. The eluting solvent was evaporated under a stream of N₂ gas at 40 °C and the sample reconstituted to 1 mL using 20:80 ACN:H₂O solvent. Filtration was done through a 0.2 µm cellulose acetate membrane filter into HPLC vials ready for analysis.

2.4 LC-ESI-MS/ MS analysis

A waters alliance 2975 liquid chromatograph (LC, Milford, MA, USA) was used for separation. An Xbridge™ (3.5 µm x 2.1 mm x 100 mm) C₁₈ reversed-phase column fitted with a Vanguard® (2.1mm x 5mm) pre-column was used. A Quattro micro mass spectrometer (MS) was used for detection. The LC solvent systems and the MS/MS instrument parameters optimized by Ngumba et al. (2016) were used for the targeted multiresidue analysis. The optimized LC-ESI-MS/MS instrument parameters for the analysis of the target compounds are shown in Table S3 of the supplementary information. Figures S1 and S2 of the supplementary information show the internal standard calibration and the matrix matched calibration graphs, respectively.

2.5 Removal efficiencies

The percentage removal efficiency (RE %) of the selected APIs from the WWTP was evaluated using Equation 1.

$$RE (\%) = \frac{(C_{Inf} - C_{Eff})}{C_{Inf}} * 100 \quad \text{Equation (1)}$$

Where C_{Inf} and C_{Eff} refer to the respective measured concentrations (µgL⁻¹) at the influent and effluent of the WWTP (Sun et al., 2015).

2.6 Risk assessment of antimicrobial resistance selection

The risk quotient (RQ) for antimicrobial resistance selection within the sampled environments was indirectly determined (Tran et al., 2019), according to the measured residual antibiotic concentrations in the representative water samples and the predicted no-effect concentration (PNEC) for resistance selection (RS) as illustrated in Equation 2. The compound-specific PNEC_(RS) values used for risk assessment were proposed by Bengtsson-Palme and Larsson (2016)

based on the EUCAST database. The $PNEC_{(RS)}$ values also factored multiple genera of pathogenic microorganisms present in the environment.

$$RQ = \frac{MEC}{PNEC_{(RS)}} \quad \text{Equation (2)}$$

MEC is the measured environmental concentration in the representative samples and $PNEC_{(RS)}$ is the compound-specific predicted no-effect concentration for resistance selection as proposed by Bengsston-Palme and Larsson (2016). The RQ results were classified as low, medium and high risk and the interpretation followed the format $RQ \geq 1$ for high risk, $1 > RQ \geq 0.1$ for medium risk and $RQ < 0.1$ for low risk (Abafe et al., 2018; Guo et al., 2016; Hanna et al., 2018).

3. Results

3.1 LC-ESI-MS/MS analysis

The results of the LC-ESI-MS/MS analysis are illustrated in Table 2. The linear correlation coefficient (r^2) values of the calibration curves was > 0.99 for all the target compounds. The limit of detection (LOD) and limit of quantification (LOQ) values varied relatively across the analytes with the majority having an $LOQ \leq 10 \text{ ngL}^{-1}$. DOX had the highest LOQ value of 135 ngL^{-1} .

Table 2: LC-ESI-MS/MS Method qualification results

Compound	RT (SD)	r^2	LOD (ngL^{-1})	LOQ (ngL^{-1})
AMO	1.77(0)	0.994	8	22
CIP	2.24 (0.08)	0.99	3	10
TMP	2.25 (0.05)	0.999	3	7
NOR	2.15 (0.06)	0.994	4	8
SMX	4.83 (0.02)	0.996	7	18
DOX	5.87 (0.01)	0.994	56	135

3.2 Prevalence of antibiotics and removal efficiency

The concentration of the targeted antibiotics in the wastewater influents, effluents, surface waters and river sediments as well as the corresponding percentage removal are shown in Table 3. The standard deviation of the measurements is shown in parenthesis. SMX was the most abundant antibiotic in all the sampling sites with values ranging from 0.03(0.01) μgL^{-1} to 56.6(4.0) μgL^{-1} . The highest value was measured in the surface water grab sample MA7, sampled approximately 200 m downstream of the effluent discharge point of WWTP1. AMO, which is a common aminopenicillin beta-lactam antibiotic, occurred at concentrations of 0.9(0.1), 0.05(0.01) and 0.3(0.1) μgL^{-1} in surface water samples MA7, MA8 and NY9, respectively. These were relatively low levels compared with the corresponding river sediment phase, in which concentrations of 4.6(0.3), 43.8(3.1), 11.7(3.2) and 7.8(1.6) μgkg^{-1} were measured for samples MA9, NY10, ME6 and ME10, respectively.

Table 3: concentrations (μgL^{-1}) of the selected antibiotics in the sampled WWTP's. AMO = Amoxicillin, CIP = Ciprofloxacin, TMP = trimethoprim, NOR = Norfloxacin, SMX = Sulfamethoxazole, and DOX = Doxycycline

	Site	Sample type	Code	AMO	CIP	TMP	NOR	SMX	DOX
Machakos	WWTP 1	Influent	MA 1	4.6(0.2) ¹	1.6(0.4)	5.6(0.1)	1.2(0.1)	49.3(2.7)	2.7(0.2)
		Effluent	MA 3	1.6(0.3)	0.4(0.3)	0.3(0.2)	0.5(0.2)	8.5(0.4)	1.5(0.4)
	Mitheu River	Surface water grab (200m upstream)	MA 4	<LOQ	1.3(0.1)	<LOQ	0.6(0.01)	49.7(1.5)	0.7(0.1)
		river sediment (200m upstream)	MA 5	n.d ³	29.3(7.2)	<LOQ	<LOQ	<LOQ	<LOQ
		Surface grab (2 km upstream)	MA 6	<LOQ	0.7(0.1)	0.2(0.1)	0.9(0.3)	0.06(0.02)	<LOQ
		Surface water grab (200m downstream)	MA 7	0.9(0.01)	0.5(0.1)	0.1(0.03)	2.2(0.4)	56.6(4.4)	<LOQ
	Mwania river	Surface grab (2 km downstream)	MA 8	0.05(0.01)	0.5(0.1)	<LOQ	0.11(0.01)	1.2(0.1)	0.3(0.1)

		River sediment (2 km downstream)	MA 9	4.6(0.3)	<LOQ	<LOQ	<LOQ	3.4(0.7)	8.2(1.3)
Nyeri county	Gatei WWTP 2	Influent	NY 1	0.2(0.06)	<LOQ	0.9(1.8)	0.9(0.1)	24.9(1.7)	<LOQ
		Effluent	NY 3	0.9(0.2)	1.8(0.2)	0.1(0.01)	2.9(0.1)	21.4(3.4)	0.7(0.01)
	Sagana river	Surface water (1km downstream)	NY 4	n.d	0.2(0.1)	<LOQ	<LOQ	n.d	n.d
		river sediment	NY 5	n.d	n.d	n.d	n.d	n.d	n.d
	Kangemi WWTP 3	Influent	NY 6	0.7(0.2)	0.8(0.1)	4.8(0.3)	2.8(0.1)	25.47(1.8)	0.4(0.1)
		Effluent	NY 8	1.24(0.3)	0.3(0.1)	0.5(0.1)	0.8(0.3)	1.3(0.4)	0.4(0.1)
	Chania river	Surface water (2m downstream)	NY 9	0.3(0.1)	n.d	<LOQ	0.1(0.03)	0.3(0.05)	n.d
		river sediment(2m downstream)	NY 10	43.8(3.1)	n.d	<LOQ	26.0(3.8)	16.3(3.9)	<LOQ
		Surface water (5m upstream)	NY 11	<LOQ	<LOQ	<LOQ	<LOQ	n.d	n.d
		river sediment (5m upstream)	NY 12	5.9(1.4)	<LOQ	1.8(0.5)	26.6(3.8)	<LOQ	32.2(5.7)
		Surface water (200m)	NY 13	n.d	<LOQ	<LOQ	0.1(0.4)	n.d	<LOQ
		river sediment	NY 14	<LOQ	35.7(4.2)	13.3(2.5)	6.6(1.4)	<LOQ	7.8(2)
Meru County	WWTP 4	Influent	ME 1	1.58(0.1)	3.0(0.7)	0.1(0.01)	1.2(0.3)	49.1(5.1)	<LOQ
		Effluent	ME 3	1.4(0.1)	2.6(0.4)	0.1(0.04)	0.8(0.1)	17(1.7)	0.5(0.1)
	Kanyuru River	river source swamp (2km upstream)	ME 5	<LOQ	0.24	n.d	n.d	<LOQ	0.1(0.01)
		river sediment (2km upstream)	ME 6	11.7(3.2)	47.4(2.8)	<LOQ	<LOQ	44.7(3.9)	<LOQ
		surface grab (500m downstream)	ME 7	n.d	0.2(0.03)	<LOQ	<LOQ	n.d	0.02(0.01)
		river sediment (500m downstream)	ME 8	<LOQ	<LOQ	<LOQ	<LOQ	10.4(1.3)	13.9(2.4)
		Surface grab (1km downstream)	ME 9	n.d	0.2(0.05)	<LOQ	<LOQ	n.d	0.1(0.03)
		river sediment(1km downstream)	ME 10	7.8(1.6)	<LOQ	<LOQ	<LOQ	<LOQ	11.4(2.1)

¹Concentration of the analytes reported in μgL^{-1} () standard deviation, n=2, ² <LOQ - Below Quantification limit ³ n.d - not detected/below limit of detection

The prevalence of the selected antibiotics was higher in the samples taken from the river sediments than in those from the surface waters. River sediment sample MA9 had SMX, AMO and DOX concentrations of 3.4(0.7), 4.6(0.3) and 8.2(1.3) μgkg^{-1} , respectively, which were considerably higher than the values of 1.2(0.1), 0.05(0.01) and 0.3(0.1) μgL^{-1} found in the surface water sampled

at the same location. Similar trend in phase distribution of the antibiotics was recorded in sediment samples NY10, NY12 and NY14 with the following concentration ranges: AMO, 5.9(1.3) to 43.8(3.1); CIP, < LOQ to 35.7(4.2); NOR, 6.6(1.4) to 26.6(3.8); DOX, 7.8(2) to 32.2(5.7) μgkg^{-1} . Sediment sample ME6, which was collected upstream of WWTP4, had higher concentration of AMO, CIP and SMX compared with downstream samples from the same site.

The removal efficiency of specific compounds at the WWTPs varied between 0 and 95%. However, higher concentrations in the effluent relative to the influent, which accounted for the negative removal efficiencies, was noted especially for AMO, CIP, NOR and DOX, as shown in Figure 2

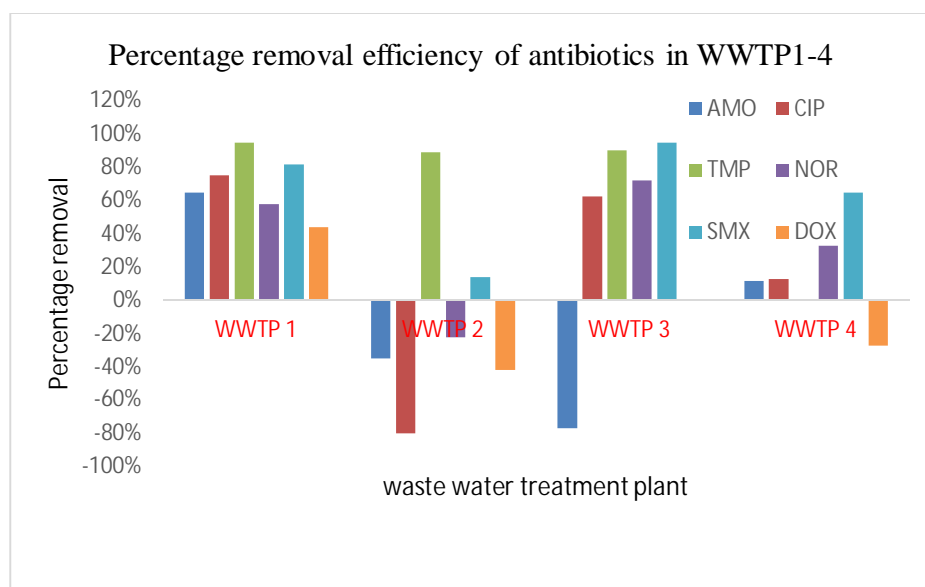


Figure 2: Percentage removal efficiencies of the antibiotics in the selected treatment plants

Generally, these findings provide evidence of environmental concentrations of residual antibiotics above their respective PNECs for resistance selection. This could signal the ineffectiveness of the existing wastewater treatment plants in removing APIs.

3.3 Risk assessment of antibiotics for resistance selection

The RQ for antibiotic resistance selection, calculated based on the compound-specific $PNEC_{(RS)}$ values as proposed by Bengtsson-Palme and Larsson (2016) are shown in Table 5. The risk of resistance selection in the aqueous phases ranged between medium and high. The high-risk figures were for AMO, NOR and CIP, with RQ values of 6.4, 5.8 and 41, respectively. Wastewater samples carried a higher risk than surface water samples, except for SMX, which exhibited a higher risk in surface water. The same compounds accounted for the increased risk of resistance selection assessed in the sediment phase. Resistance selection was one to two times more likely to occur in the wastewater and river sediment phases than in the surface water.

Table 5: Concentrations and risk quotient for resistance selection for the selected antibiotics in Kenya. MEC=Measured Environmental Concentrations, PNEC=Predicted no effect concentration for resistance selection as proposed by Bengtsson-Palme and Larsson (2016). AMO = Amoxicillin, CIP = Ciprofloxacin, TMP = trimethoprim, NOR = Norfloxacin, SMX = Sulfamethoxazole, and DOX = Doxycycline.

API	MEC (μgL^{-1}) This study		PNEC (RS) (μgL^{-1}) ^a (resistance selection)	Covered genera ^b (families)	Risk quotient (resistance selection)
	Effluent	surface water			
AMO	0.9-1.6	0.05-0.9	0.25	19(12)	0.2-6.4 (medium - high)
NOR	0.5-2.9	0.1-2.2	0.5	12(8)	0.2-5.8 (medium -high)
TMP	0.1-0.5	0.1-0.2	0.5	15(7)	0.2 -1 (medium - high)
CIP	0.43-2.6	0.2-1.3	0.064	29(18)	3.1 - 40.6 (high)
SMX	1.3-21.4	0.1-56.6	16	6(4)	<0.1 - 3.53 (low-high)
DOX	0.4-1.5	0.1-0.7	2	20(11)	<0.1 - 0.7 (low-medium)

^aPNEC value corresponds to the size-adjusted lowest MIC divided by an assessment factor of 10 as proposed by Bengtson-palme and Larsson (2016).

^bThe number of different bacterial genera and families tested against the specific antibiotic.

4. Discussion

4.1 fate of antibiotics in the natural environment

The MEC for the analyzed antibiotics mostly occurred in low concentrations in the aqueous samples as compared with the sediment samples. The low levels of AMO in the aqueous phase may be attributed to the fact that beta-lactams are relatively hydrophobic, tend to migrate to the sediment phase and are generally highly susceptible to hydrolysis either by chemical or enzymatic agents (Hirte et al., 2016).

TMP was mostly detected in wastewater influent and river sediment, but infrequently in the surface water. TMP-SMX combinations are used to treat broad spectrum infections including cholera. They are also administered to immunosuppressed patients as prophylaxis against opportunistic infections (Kronbichler et al., 2018; Walker et al., 2010).

At one of the four sampling sites, the concentration of target compounds in river water upstream and downstream of this treatment plant (WWTP1) was considerably higher than the concentration in the influent and effluent. This could be attributed to direct discharge of untreated wastewater into water bodies, taking into account that less than 10% of the population in these areas are connected to the centralized sewage treatment system. Intentional tampering and blockage of the sewer line en route to the plant was noted. This was done to divert the sewerage water into the river for vegetable farming along the river banks. It is highly likely that this directly contributed

to the higher levels of the pharmaceutical compounds in the river samples compared to those found in the plant effluent samples.

The concentration levels determined in the sediment samples indicated accumulation of some of the antibiotics, mostly SMX and DOX, in the sediment phase as compared to the aqueous phase. The higher measured concentrations in the sediment samples could mean that residual antibiotics exert higher selection pressure within the sediment phase than in the aqueous phase.

The irregular flux in environmental concentrations of antibiotics between the influent, effluent, surface water and river sediments could be attributed to hydrological flow conditions. During dry seasons, the concentration could be higher, and vice versa for wet seasons due to dilution. This automatically influences chemical and biological reactions within the natural environment. Waste stabilization ponds, such as those sampled in this study, have limited ability to remove recalcitrant organic matter (Ignatev and Tuhkanen, 2019). Accumulation could be the result of the sludge being removed with irregular frequency, as well as the resuspension of the adsorbed APIs in the sludge, especially when decomposition occurs in well aerated conditions (Ho et al., 2017).

Negative removal efficiencies for APIs have been reported (K'oreje et al., 2018; Li et al., 2009; Polesel et al., 2016; Thiebault et al., 2017; Udert et al., 2015). Factors causing this may include elimination of antibiotics adsorbed into the particulate matter during sample processing and unaccounted-for hydraulic retention time during sampling. Physicochemical changes during the treatment process influence the adsorption behavior of the antibiotics and hence affect the partition ratio between the aqueous, suspended and sediment phases, and between the influent and effluent concentration (Lindberg et al., 2005). API accumulation, biotic or abiotic dissolution, as well as back transformation and de-conjugation of metabolic products back to parent compounds, can all lead to increased measured concentrations in the effluent relative to the influent (Archer et al.,

2017; Haddad et al., 2015; Polesel et al., 2016). SMX transformational products have been shown to back transform to the parent compound under biological and photolytic degradation conditions (Archer et al., 2017; Bagnis et al., 2020). Previous studies in Kenya have reported the presence of 14-112 μgL^{-1} of SMX and 4-20 μgL^{-1} of TMP in wastewater influent, and 10 μgL^{-1} of SMX in the effluent (K'oreje et al., 2018). In addition, two independent studies of the Nairobi river surface water reported SMX concentrations of 13.76 μgL^{-1} (Ngumba et al., 2016) and 23.35 μgL^{-1} (K'oreje et al., 2012) and TMP concentrations 2.65 μgL^{-1} (Ngumba et al., 2016) and 9.48 μgL^{-1} (K'oreje et al., 2012), respectively. In this article, we report values of the same order of magnitude as other Kenyan studies, but considerably higher than those reported in the global North, as shown in Table 4.

Table 4. Previous observations of antibiotic concentrations (μgL^{-1}) in surface waters and urban lakes in different countries and regions. AMO = Amoxicillin, CIP = Ciprofloxacin, TMP = trimethoprim, NOR = Norfloxacin, SMX = Sulfamethoxazole, and DOX = Doxycycline.

Location	Sample type	AMO	CIP	TMP	NOR	SMX	Ref
Nairobi,kenya	river water	n.r	0.509	2.65	nr	13.765	(Ngumba et al., 2016)
Nairobi,kenya	river water	n.r	0.168	3.346	n.r	11.25	(Bagnis et al., 2020)
Nairobi,kenya	river water	n.r	n.r	9.48	nr	23.35	(K'oreje et al., 2012b)
Hanoi, Vietnam ^a	surface water	<LOQ - 1.126	<LOQ - 0.115	0.002-0.07	n.a	0.11 - 3.5	(Tran et al., 2019)
Africa ^a	surface water	n.r	nd -0.51	0.024 -6.95		nd - 13.8	(Madikizela et al., 2017)
Global ^b	surface water	n.r	18.99	0.037	3.457	0.095	(aus der Beek et al., 2016)

							(aus der Beek et al., 2016)
Europe ^b	surface waters	n.r	0.002	0.012	0.004	0.033	

a = Concentration range, b = average concentration, n.r = Not reported

4.2 risk of evolution of antimicrobial resistance

Various studies have been conducted on environmental pollution by pharmaceuticals and personal care products (Fatta-Kassinos et al., 2011). However, less attention has been given to the risk associated with the development and propagation of antimicrobial resistant bacteria and genes in the hydrological cycles as a result of residual antibiotics. Besides their effect on larger aquatic organisms, their impact on pathogenic bacteria, especially the selection of resistant strains, is of great concern.

Generally, most of the antibiotics were measured above their compound-specific PNEC values for resistance selection. Increased prevalence of antibiotic resistant bacteria could be a result of environmental bacterial communities undergoing resistance selection pressure due to continuous contact with residual antibiotics (Michael et al., 2013; Wu et al., 2018). It has been predicted that resistance resulting from bacterial exposure to subinhibitory concentrations of antibiotics is irreversible, even in the absence of the antibiotic, since the mutants are more stable than the bacteria selected at higher concentrations (Sandegren, 2014). Enrichment and selective advantage of resistant bacteria has also been confirmed at subinhibitory concentrations (Gullberg et al., 2011; Liu et al., 2011).

The use of untreated wastewater for agricultural purposes was observed during sampling. This potentially creates an enormous biosecurity risk by exposing the environment and food chain to residual APIs. Antimicrobial resistance can be transmitted to humans and animals through the food

chain, by consumption of untreated water, or indirectly through environmental emissions. Mortality due to drug resistant bacterial infections, like tuberculosis, is on the rise in Kenya, with approximately 169 000 deaths reported in 2017, 30% of which were attributed to multi-drug resistant bacteria (WHO, 2017). Furthermore, 36.7% multidrug resistance among *Klebsiella spp* strains has been reported on the central and western regions of Kenya (Taitt et al., 2017). Further research into AMR at the studied sites is needed.

5. Conclusions

This study presents a risk assessment of the prevalence and resistance selection of six antibiotics (AMO, NOR, CIP, DOX, TMP and SMX), in the wastewaters, surface waters and river sediments of four Kenyan wastewater treatment plants. Levels ranging from <0.1 to $56.6 \mu\text{gL}^{-1}$ were found, which are comparable to values reported in other parts of Kenya, and two to three orders of magnitude higher than data reported in the global North.

Presence of APIs in the sediment phase were also reported in this study. The findings present a broader picture of the situation in two previously unexplored, relatively smaller counties besides Nairobi and Kisumu, which have been studied previously. Low connectivity to a centralized wastewater treatment network ($<10\%$) could be the biggest driver directing discharge of untreated waste into the water bodies.

In most cases, the antibiotic levels reported in this study were higher than the PNEC values for resistance selection for multiple genera of pathogenic bacteria. This implies a medium to high risk of selection for antibiotic resistance within the respective environmental compartments, a major threat to human health.

Data presented in this paper from previously unexplored areas can help to improve the knowledge and risk assessment of the levels of active antibiotics in the aqueous and sediment phases in Kenyan waters. Based on this data, we recommend raising general public awareness of the possible dangers of directly discharging human waste into water bodies. Local authorities in the study areas are encouraged to increase access to sustainable sanitation solutions in order to mitigate the direct discharge of wastewater into water bodies, especially within informal settlements. This information will help healthcare stakeholders and policymakers to understand the possible sources and drivers of antibiotic resistance within natural environments. It will also be beneficial in the process of formulating strategies to mitigate antimicrobial resistance.

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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SUPPLEMENTARY MATERIAL

530

Occurrence of antibiotics and risk of antibiotic resistance evolution in selected Kenyan

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wastewaters, surface waters and sediments

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547 **Table S1:** sample collection sites

	Site	Sample type	Sample Code
Machakos	WWTP 1	Influent	MA 1
		Effluent	MA 3
	Mitheu River	Surface water grab (200m upstream)	MA 4
		river sediment (200m upstream)	MA 5
		Surface grab (2 km upstream)	MA 6
		Surface water grab (200m downstream)	MA 7
	Mwania river	Surface grab (2 km downstream)	MA 8
		River sediment (2 km downstream)	MA 9
Nyeri county	Gatei WWTP 2	Influent	NY 1
		Effluent	NY 3
	Sagana river	Surface water grab (1km downstream)	NY 4
		river sediment	NY 5
	Kangemi WWTP 3	Influent	NY 6
		Effluent	NY 8
	Chania river	Surface grab (2m downstream)	NY 9
		river sediment(2m downstream)	NY 10
		Surface water grab (5m upstream)	NY 11
		river sediment (5m upstream)	NY 12
		Surface water grab (200m)	NY 13
		river sediment	NY 14
Meru County	WWTP 4	Influent	ME 1
		Effluent	ME 3
	Kanyuru River	river source swamp (2km upstream)	ME 5
		river sediment (2km upstream)	ME 6
		surface grab (500m downstream)	ME 7
		river sediment	ME 8
		Surface grab downstream 1km	ME 9
		river sediment	ME 10

548

549 **Table S2:** physicochemical properties of selected API's

compound	¹ molecular formula	¹ CAS No.	² water solubility mgL ⁻¹	² Excretion as parent compound (%)	³ log K _{ow}
Doxycycline (DOX)	C ₂₂ H ₂₄ N ₂ O ₈	564-25-0	630	70	
Amoxicillin (AMO)	C ₁₆ H ₁₉ N ₃ O ₅ S	26787-78-0	958	60-80	
Sulfamethoxazole(SMX)	C ₁₀ H ₁₁ N ₃ O ₃ S	723-46-6	610	15-25	0.89
			400	80-90	0.91
Trimethoprim (TMP)	C ₁₄ H ₁₈ N ₄ O ₃	738-70-5			
	C ₁₇ H ₁₈ FN ₃ O ₃		80	80	0.28
Ciprofloxacin (CIP)		85721-33-1			
Norfloxacin (NOR)	C ₁₆ H ₁₈ N ₃ O ₃ F	70458-96-7	13500	60	

¹ Drugbank www.drugbank.ca ² Ngumba et al., 2016b ³ Madikizela et al., 2017

Table S3: Optimized LC-ESI-MS/MS instrument parameters for the analysis of the target compounds

Target compound	ILIS ^a	RT (Sd) ^b	Precursor ion [M+H] ⁺ (m/z)(CV) ^c	Quantifier ion (m/z) (CE) ^d	Qualifier ion (CE)
TET	n.a	2.63 (0.13)	445.0 (25)	154.0 (25)	410.0 (20)
AMO	n.a	1.77	365.9 (15)	113.9 (19)	348.9 (9)
CIP	[² H ₈]-CIP	2.24 (0.08)	332.1 (34)	288.0 (19)	314.1 (19)
TMP	[² H ₉]-TMP	2.25 (0.05)	291.1 (34)	123.0 (19)	230.0 (19)
NOR	[² H ₈]-NOR	2.15 (0.06)	320.3 (30)	276.0 (18)	302.0 (25)
SMX	[² H ₄]-SMX	4.83 (0.02)	254.0 (28)	156.0 (18)	108.0(17)
DOX	n.a	5.87 (0.01)	445.4 (30)	428.0 (25)	410.1 (25)

^aILIS isotopically labelled internal standard. ^bRT retention time. ^cCV collision voltage ^dCE collision energy.
n.a not available¹

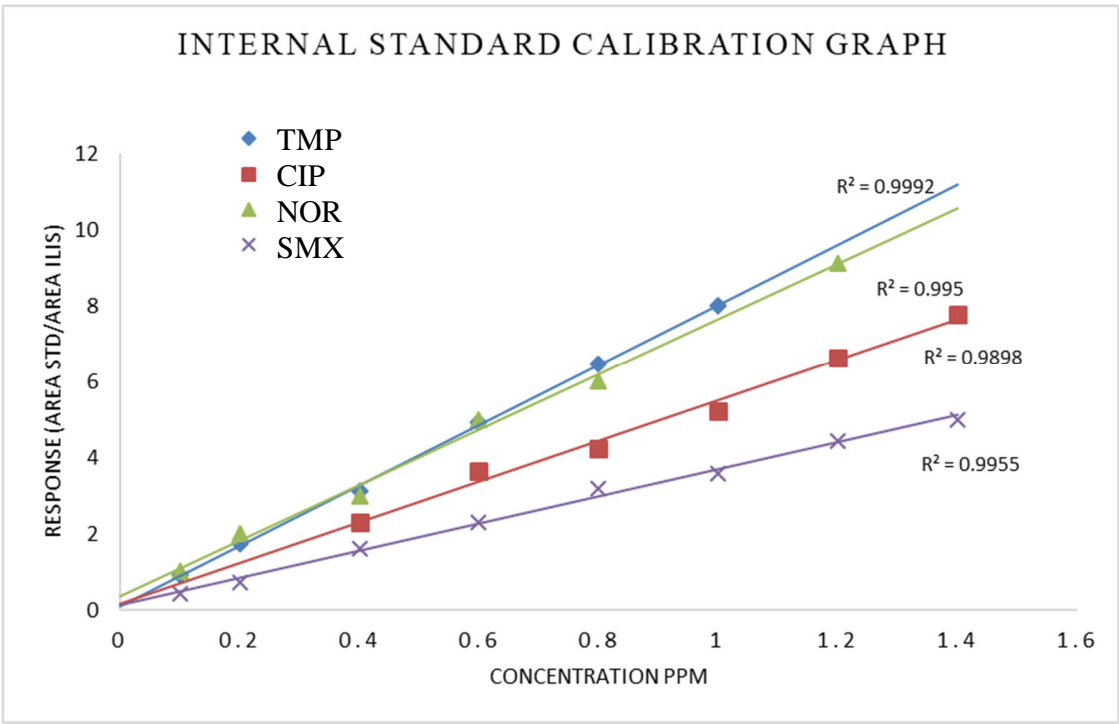


Figure S1: Calibration graph for TMP, CIP, NOR and SMX constructed by plotting the ratio of the Area of the standard divided by the area of the isotopically labelled internal standard against the concentration.

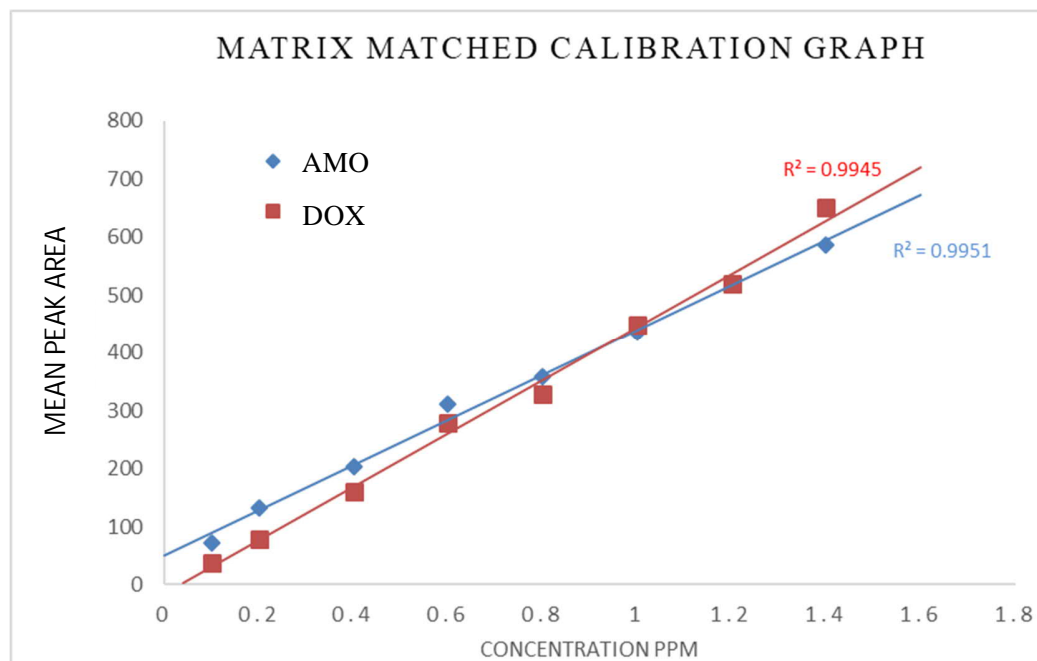


Figure S2: Matrix matched calibration graph for AMO and DOX constructed by spiking surface water at concentration levels between 0ppm (blank) and 1.4ppm

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